

Fourth Edition

Antimicrobial Therapy

in Veterinary Medicine

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Preface

It has now been almost 20 years since publication of the first edition of *Antimicrobial Therapy in Veterinary Medicine*. The growth of knowledge in the area of anti-infective chemotherapy in recent years has made it challenging to compile a comprehensive text in approximately 600 pages. For this new edition, two additional editors, Steeve Giguère and Patricia M. Dowling, have joined the editorial team already in place, with Steeve Giguère as major editor. The fourth edition also welcomes 23 new contributors. We are grateful to all the contributors for the care and effort they have put into their chapters.

The fourth edition is a restructured, completely updated, and considerably expanded version of the third edition. The book is now divided in four sections. The first section provides general principles of antimicrobial therapy and includes a new chapter on the pharmacokinetic-pharmacodynamic relationship of antimicrobial agents. The second section describes each class of antimicrobial agents, with emphasis on information specific to veterinary species. It has been revised to include antimicrobial agents recently developed for use in humans or in veterinary medicine. The third section deals with special considerations and in-

cludes new chapters on prophylactic use of antimicrobial agents, antimicrobial chemotherapy for the neutropenic patient, and prudent use of antimicrobial agents. Chapters on regulation of antibiotic use in animals, growth promotion uses of antimicrobial agents, and antimicrobial drug residues in foods of animal origin have been revised against the background of extensive reexamination in many countries of the use of antimicrobial agents as growth promoters or in the prevention of disease in animals. The fourth section addresses the specific principles of antimicrobial therapy in multiple veterinary species. A chapter on antimicrobial therapy in New World camelids has been added to the fourth edition to reflect the increase in popularity of these species.

We thank the staff of Blackwell Publishing, particularly Dede Andersen, Carrie Sutton, and Blaire McPherson, for their help, patience, and support of this book. We encourage readers to send comments or suggestions for improvements to Steeve Giguère so that future editions can be improved.

Steeve Giguère, John Prescott, Desmond Baggot,
Robert Walker, Patricia Dowling

Important Notice

The indications and dosages of all drugs in this book are the recommendations of the authors and do not always agree with those given on package inserts prepared by pharmaceutical manufacturers in different countries. The medications described do not necessarily have the specific approval of national regulatory authorities, including the US Food and Drug Administration, for use in the diseases and dosages recom-

mended. In addition, while every effort has been made to check the contents of this book, errors may have been missed. The package insert for each drug product should therefore be consulted for use, route of administration, dosage, and (for food animals) withdrawal period, as approved by the reader's national regulatory authorities.

Abbreviations

Abbreviations used in this book include:

MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
PO	Per os, oral administration
IM	Intramuscular administration
IV	Intravenous administration
SC	Subcutaneous administration
SID	Single daily administration
BID	Twice daily administration (every 12 hours)
TID	Three times daily administration (every 8 hours)
QID	Four times daily administration (every 6 hours)
q 6h, q 8h, q 12h, etc.	Every 6, 8, 12 hours, etc.

For example, a dosage of “10 mg/kg TID IM” means 10 milligrams of the drug per kilogram of body weight, administered every 8 hours intramuscularly.

Section I

General Principles of Antimicrobial Therapy

Antimicrobial Drug Action and Interaction: An Introduction

Steeve Giguère

Antimicrobial drugs exploit differences in structure or biochemical function between host and parasite. Modern chemotherapy is traced to Paul Ehrlich, a pupil of Robert Koch, who devoted his career to discovering agents that possessed selective toxicity so that they might act as so-called “magic bullets” in the fight against infectious diseases. The remarkable efficacy of modern antimicrobial drugs still retains a sense of the miraculous. Sulfonamides, the first clinically successful broad-spectrum antibacterial agents, were produced in Germany in 1935.

However, it was Fleming’s 1929 discovery of the antibiotic penicillin, a fungal metabolite, and its subsequent development by Chain and Florey during World War II that led to the antibiotic revolution. Within a few years of the introduction of penicillin, many other antibiotics were described. This was followed by the development of semisynthetic and synthetic (e.g., sulfonamides and fluoroquinolones) antimicrobial agents, which has resulted in an increasingly powerful and effective array of compounds used to treat infectious diseases. In relation to this, the term *antibiotic* has been defined as a low molecular weight substance produced by a microorganism that at low concentrations inhibits or kills other microorganisms. In contrast, the word *antimicrobial* has a broader definition than antibiotic and includes any substance of natural, semisynthetic, or synthetic origin that kills or inhibits the growth of a microorganism but causes little or no damage to the host. Thus an antibiotic is an antimicrobial agent, though the converse may not be true. However, in many publications “antimicrobial agent” is synonymous with “antibiotic”.

The marked structural and biochemical differences

between prokaryotic and eukaryotic cells give antimicrobial agents greater opportunities for selective toxicity against bacteria than against other microorganisms such as fungi, which are nucleated like mammalian cells, or viruses, which require their host’s genetic material for replication. Nevertheless, in recent years increasingly effective antifungal and antiviral drugs have been introduced into clinical practice.

Important milestones in the development of antibacterial drugs are shown in Figure 1.1. The therapeutic use of these agents in veterinary medicine has usually followed their use in human medicine because of the enormous costs of development. However, some antibacterial drugs have been developed specifically for animal health and production (e.g., tylosin, tiamulin, tilmicin, ceftiofur, tulathromycin). Figure 1.1 highlights the relationship between antibiotic use and the development of resistance in many target microorganisms.

Spectrum of Activity of Antimicrobial Drugs

Antimicrobial drugs may be classified in a variety of ways, based on three basic features.

Class of Target Microorganism

Antiviral and antifungal drugs generally are active only against viruses and fungi, respectively. Antibacterial agents are described as *narrow-spectrum* if they inhibit only bacteria or *broad-spectrum* if they also inhibit mycoplasma, rickettsia, and chlamydia. The spectrum of activity of common antibacterial agents is shown in Table 1.1.

Human Infectious Diseases

Antibacterial Agents

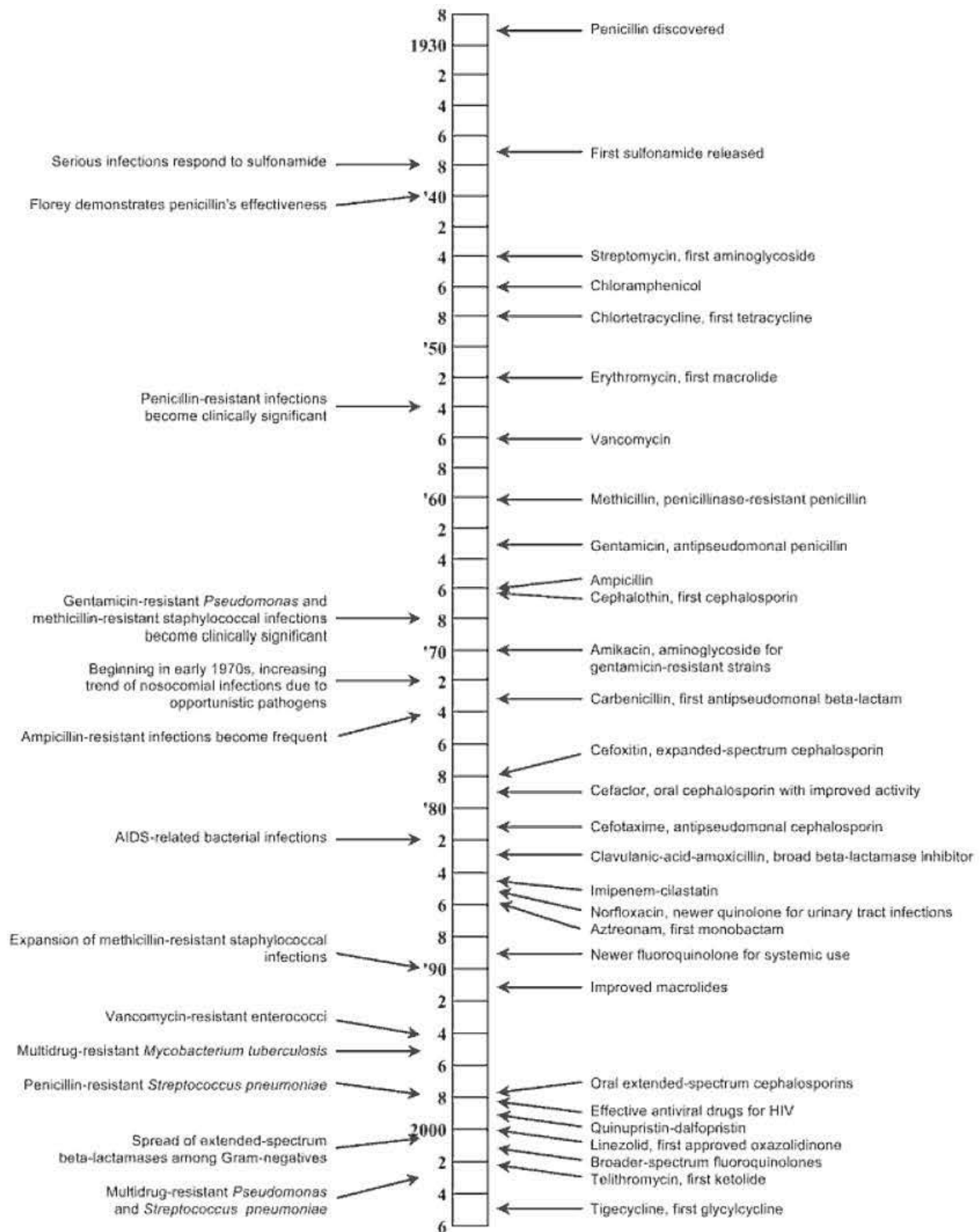


Figure 1.1. Milestones in human infectious disease and their relationship to development of antibacterial drugs. Modified and reproduced with permission from Kammer (1982).

Table 1.1. Spectrum of activity of common antibacterial drugs.

Drug	Class of Microorganism					
	Bacteria	Fungi	Mycoplasma	Rickettsia	Chlamydia	Protozoa
Aminoglycosides	+	—	+	—	—	—
Beta-lactams	+	—	—	—	—	—
Chloramphenicol	+	—	+	+	+	—
Fluoroquinolones	+	—	+	+	+	—
Lincosamides	+	—	+	—	—	+/-
Macrolides	+	—	+	—	+	+/-
Oxazolidinones	+	—	+	—	—	—
Pleuromutilins	+	—	+	—	+	—
Tetracyclines	+	—	+	+	+	+/-
Streptogramins	+	—	+	—	+	+/-
Sulfonamides	+	—	+	—	+	+
Trimethoprim	+	—	—	—	—	+

+/- Activity against some protozoa

Antibacterial Activity

Some antibacterial drugs are also considered narrow-spectrum in that they inhibit only Gram-positive or Gram-negative bacteria, whereas broad-spectrum drugs inhibit both Gram-positive and Gram-negative bacteria. However, this distinction is not always absolute as some agents may be primarily active against Gram-positive bacteria but will also inhibit some Gram-negatives (Table 1.2). With the greater availabil-

ity of broad-spectrum antibacterial drugs, these terms are increasingly uncommon.

Bacteriostatic or Bactericidal Activity

Some antibacterial agents inhibit the growth of a bacterium at one concentration, the minimum inhibitory concentration (MIC) (e.g., 0.25 µg/ml), but require a higher concentration to kill it, the minimum bactericidal concentration (MBC) (e.g., 4.0 µg/ml). An anti-

Table 1.2. Antibacterial activity of selected antibiotics.

Spectrum	Aerobic Bacteria		Anaerobic Bacteria		Examples
	Gram +	Gram —	Gram +	Gram —	
Very broad	+	+	+	+	Carbapenems; chloramphenicol; 3rd-generation fluoroquinolones; glycyclcyclines
Intermediately broad	+	+	+	(+)	3rd- and 4th-generation cephalosporins
	+	(+)	+	(+)	2nd generation cephalosporins
	(+)	(+)	(+)	(+)	Tetracyclines
Narrow	+	+/-	+	(+)	Ampicillin; amoxicillin; 1st-generation cephalosporins
	+	—	+	(+)	Penicillin; lincosamides; glycopeptides; streptogramins; oxazolidinones
	+	+/-	+	(+)	Macrolides
	+/-	+	—	—	Monobactams; aminoglycosides
	(+)	+	—	—	2nd-generation fluoroquinolones
	(+)	(+)	—	—	Trimethoprim-sulfa
	—	—	+	+	Nitroimidazoles
	+	—	(+)	(+)	Rifamycin

+ Excellent activity
 (+) Moderate activity
 +/- Limited activity
 — No or negligible activity

bacterial agent that exhibits a large dilution difference between inhibitory and cidal effects is considered to be a bacteriostatic drug. On the other hand, an antibacterial agent that kills the bacterium at or near the same drug concentration that inhibits its growth is considered to be a bactericidal drug. Under certain clinical conditions this distinction is important but it is not absolute. In other words, some drugs are often bactericidal (e.g., beta-lactams, aminoglycosides) and others are usually bacteriostatic (e.g., chloramphenicol, tetracyclines), but this distinction is an approximation, depending on both the drug concentration at the site of infection and the microorganism involved. For example, benzyl penicillin is bactericidal at usual therapeutic concentrations and bacteriostatic at low concentrations.

Time or Concentration Dependent Activity

Antimicrobial agents are often classified as exerting either time-dependent or concentration-dependent activity depending on their pharmacodynamic properties. The pharmacodynamic properties of a drug address the relationship between drug concentration and antimicrobial activity (Chapter 5). Drug pharmacokinetic features, such as serum concentrations over time and area under the serum concentration-time curve (AUC), when integrated with MIC values, can predict the probability of bacterial eradication and clinical success. These pharmacokinetic and pharmacodynamic relationships are also important in preventing the selection and spread of resistant strains.

The most significant factor determining the efficacy of beta-lactams, macrolides, tetracyclines, trimethoprim-sulfonamide combinations, chloramphenicol, and glycopeptides is the length of time that serum concentrations exceed the MIC of a given pathogen. Increasing the concentration of the drug several-fold above the MIC does not significantly increase the rate of microbial killing. Rather, it is the length of time that bacteria are exposed to concentrations of these drugs above the MIC that dictates their rate of killing. Optimal dosing of such antimicrobial agents involves frequent administration.

Other antimicrobial agents, such as the aminoglycosides, fluoroquinolones, and metronidazole, exert concentration-dependent killing characteristics. Their rate of killing increases as the drug concentration increases above the MIC for the pathogen, and it is not necessary or even beneficial to maintain drug levels

above the MIC between doses. Thus, optimal dosing of aminoglycosides and fluoroquinolones involves administration of high doses at long dosing intervals.

Some drugs exert characteristics of both time- and concentration-dependent activity. The best predictor of efficacy for these drugs is the 24-hour AUC/MIC ratio. Glycopeptides, rifampin and, to some extent, fluoroquinolones fall within this category (Chapter 5).

Mechanisms of Action of Antimicrobial Drugs

Antibacterial Drugs

Figure 1.2 summarizes the diverse sites of action of the antibacterial drugs. Their mechanisms of action fall into five categories: inhibition of cell wall synthesis, damage to cell membrane function, inhibition of nucleic acid synthesis or function, inhibition of protein synthesis, and inhibition of folic acid synthesis.

Antibacterial drugs that affect cell wall synthesis (beta-lactam antibiotics, bacitracin, vancomycin) or inhibit protein synthesis (aminoglycosides, chloramphenicol, lincosamides, macrolides, streptogramins, pleuromutilins, tetracyclines, oxazolidinones) are more numerous and important than those that affect cell membrane function (polymyxins) or nucleic acid function (nitroimidazoles, nitrofurans, quinolones, rifampin), although the development of fluoroquinolones has been a major recent advance in antimicrobial therapy. Agents that affect folic acid synthesis (sulfonamides, trimethoprim) have greater selective toxicity than those that affect nucleic acid synthesis.

Searching for New Antibacterial Drugs

Most antibacterial drugs in major use are analogs of only six major classes of antibiotics (aminoglycosides, cephalosporins, macrolides, penicillins, quinolones, and tetracyclines). The problem of increasing resistance drives efforts to find novel antibiotics, particularly narrow-spectrum antibiotics with activity against defined pathogens. The approaches in the search for novel antibiotics include: further development of analogs of existing agents; identifying novel targets based on a biotechnological approach, including use of information obtained from bacterial genome sequencing and gene cloning; screening of natural products from plants and microorganisms from unusual ecological niches other than soil; development of antibacterial peptide molecules derived

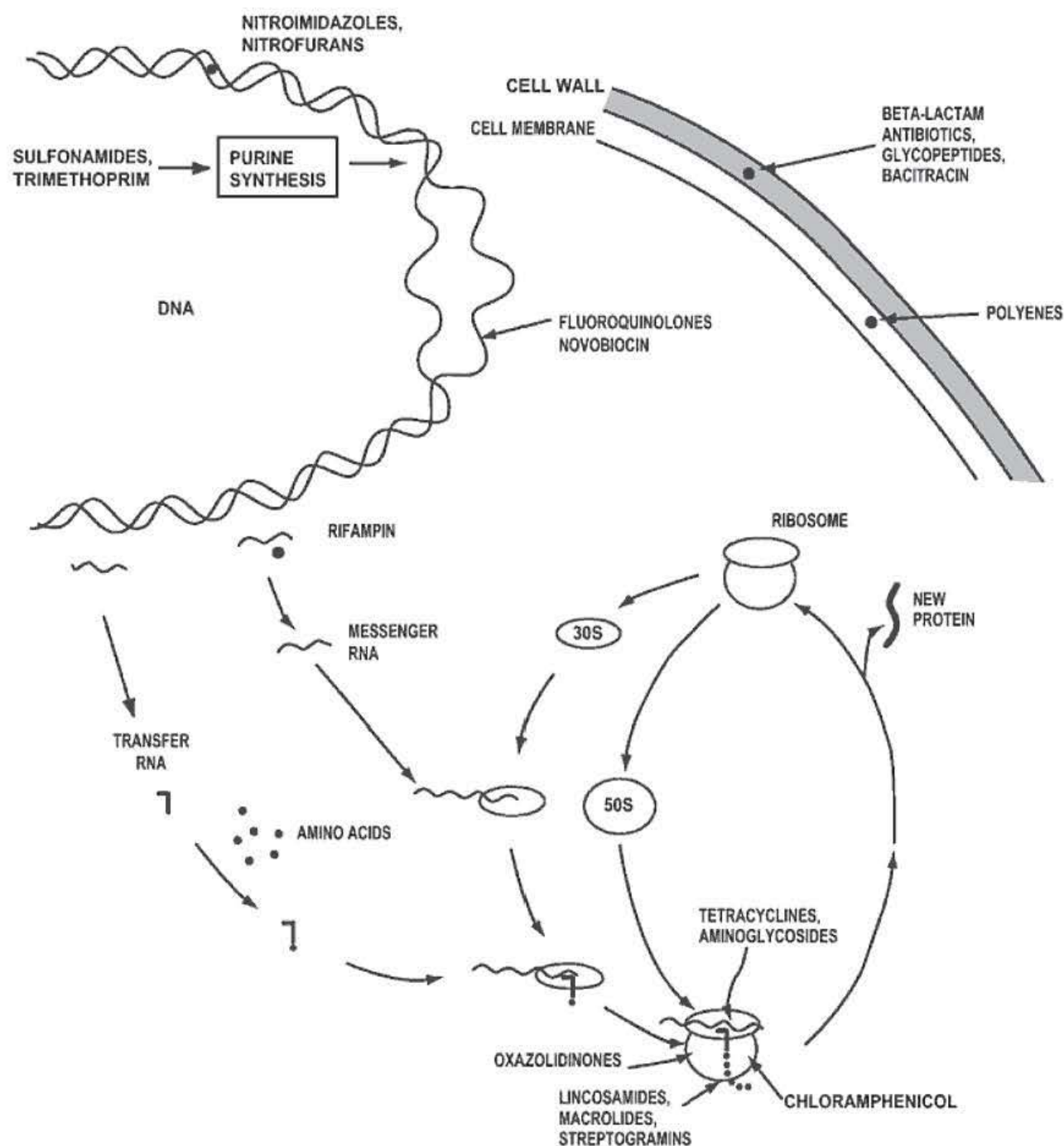


Figure 1.2. Sites of action of commonly used antibacterial drugs. Modified and reproduced with permission after Aharonowitz and Cohen (1981).

from phagocytic cells of many species; screening for novel antimicrobials using combinatorial chemical libraries; development of synthetic antibacterial drugs with novel activities, such as oxazolidinones; renewed development of antibiotic classes which were abandoned early in the antibiotic revolution because there

were existing drug classes with similar activities; development of “chimeramycins” by laboratory recombination of genes encoding antibiotics of different classes; and combination of antibacterial drugs with iron-binding chemicals targeting bacterial iron uptake mechanisms.

Antifungal Drugs

Most currently used systemic antifungal drugs (polyenes, azoles) damage cell membrane function by binding ergosterols that are unique to the fungal cell membrane (Chapter 19). The AIDS epidemic and the expansion of transplant technology have resulted in increased numbers of immunosuppressed individuals in many societies. Their susceptibility to fungal infections has renewed interest in the discovery and development of new antifungal agents. The focus of antifungal drug development has shifted to cell wall structures unique to fungi (1,3- β -D-glucan synthase inhibitors, chitin synthase inhibitors, mannoprotein binders) (Figure 19.1).

Antiviral Drugs

Antiviral drugs act only during viral replication; newer analogs are targeted at inhibition of absorption or penetration of viruses into the cell or inhibition of their assembly and release (Figure 20.1). The distinction between deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) viruses is important in antiviral therapy. The AIDS crisis has led to a dramatic increase in discovery and development of new, clinically effective antiviral drugs.

Antibacterial Drug Interactions: Synergism, Antagonism, and Indifference

Knowledge of the different mechanisms of action of antimicrobials provides some ability to predict their interaction when they are used in combination. It was clear from the early days of their use that combinations of antibacterials might give antagonistic rather than additive or synergistic effects. Concerns regarding combinations include the difficulty in defining synergism and antagonism, particularly their method of determination *in vitro*; the difficulty of predicting the effect of a combination against a particular organism; and the uncertain clinical relevance of *in vitro* findings. The clinical use of antimicrobial drug combinations is described in Chapter 6. With the availability of broad-spectrum antibacterial drugs, combinations of these drugs are less commonly used, except for specific purposes.

An antibacterial combination is *additive* or *indifferent* if the combined effects of the drugs equal the sum of their independent activities measured separately;

synergistic if the combined effects are significantly greater than the independent effects; and *antagonistic* if the combined effects are significantly less than their independent effects. Synergism and antagonism are not absolute characteristics. Such interactions are often hard to predict, vary with bacterial species and strains, and may occur only over a narrow range of concentrations or ratios of drug components. Because antimicrobial drugs may interact with each other in many different ways, it is apparent that no single *in vitro* method will detect all such interactions. Although the techniques to detect and quantify interactions are relatively crude, the observed interactions occur clinically.

The two methods commonly used, the *checkerboard* and the *killing curve* methods, measure two different effects (growth inhibition and killing, respectively). Again, clinical and laboratory correlation is sometimes poor. In the absence of simple methods for detecting synergism or antagonism, the following general guidelines may be used.

Synergism of Antibacterial Combinations

Antimicrobial combinations are frequently synergistic if they involve: (1) sequential inhibition of successive steps in metabolism (e.g., trimethoprim-sulfonamide); (2) sequential inhibition of cell wall synthesis (e.g., mecillinam-ampicillin); (3) facilitation of one antibiotic's entry by another (e.g., beta-lactam-aminoglycoside); (4) inhibition of inactivating enzymes (e.g., ampicillin-clavulanic acid); and (5) prevention of emergence of resistant populations (e.g., erythromycin-rifampin).

Antagonism of Antibacterial Combinations

Some instances of antagonism in antibacterial combinations are more laboratory artifact than clinical reality. However, there have been only a few well-documented situations where antagonism is clinically important. Antagonism may occur if antibacterial combinations involve: (1) inhibition of bactericidal activity, such as treatment of meningitis in which a bacteriostatic drug prevents the bactericidal activity of another; (2) competition for drug-binding sites, such as macrolide-chloramphenicol combinations (of uncertain clinical significance); (3) inhibition of cell permeability mechanisms, such as chloramphenicol-aminoglycoside combinations (of uncertain clinical significance); and (4) induction of beta-lactamases by

beta-lactam drugs, such as imipenem and ceftiofur combined with older beta-lactam drugs that are beta-lactamase unstable.

The impressive complexity of the interactions of antibiotics, the differences of such effects among bacterial species, and the uncertain applicability of *in vitro* findings to clinical settings make predicting the effects of some combinations hazardous. For example, the same combination may cause both antagonism and synergism in different strains of the same pathogen. Laboratory determinations are critical, but may give conflicting results depending on the test used. In the absence of other guidelines, mechanisms of action are probably the best predictors of the outcome of antibiotic interactions.

In general, the use of combinations should be avoided, because (1) the toxicity of the antibiotics will be at least additive and may be synergistic, (2) the ready availability of broad-spectrum bactericidal drugs has made combined use largely unnecessary, and (3) poor combinations may lead to bacterial superinfection. There are, however, well-established circumstances, discussed in Chapter 6, in which combinations of drugs are more effective and often less toxic than drugs administered alone.

Bibliography

- Aharonowitz Y, Cohen G. 1981. The microbiological production of pharmaceuticals. *Sci Am* 245:141.
- Bryskier A. 2005. In pursuit of new antibiotics. In: Bryskier A (ed). *Antimicrobial agents: antibacterials and antifungals*. Washington: ASM Press.
- Hirsh DC, et al. 1990. Lack of supportive data for use of ampicillin together with trimethoprim-sulfonamide as broad-spectrum antimicrobial treatment of bacterial disease in dogs. *J Am Vet Med Assoc* 197:594.
- Kammer RB. 1982. Milestones in antimicrobial therapy. In: Morin RB, Gorman M, (eds). *Chemistry and Biology of Beta-Lactam Antibiotics*. Vol 3. Orlando, FL: Academic Press.
- Pillai SK, et al. 2005. Antimicrobial combinations. In: Lorian, V (ed). *Antibiotics in Laboratory Medicine*, 5th Edition. Philadelphia: Lippincott Williams and Wilkins.
- Ryback MJ, McGrath BJ. 1996. Combination antimicrobial therapy for bacterial infections. *Drugs* 52:390.
- Silen JY, et al. 1998. Screening for novel antimicrobials from encoded combinatorial libraries by using a two-dimensional agar format. *Antimicrob Agents Chemother* 42: 1447.

Antimicrobial Susceptibility Testing Methods and Interpretation of Results

Robert D. Walker

Veterinary diagnostic microbiology is a specialty area within the overall field of microbiology. Although the majority of the isolation and identification techniques are comparable to those used for human pathogens, there are many veterinary pathogens that require unique cultivation or identification procedures and conditions (Watts and Yancey, 1994). This also applies to the interpretation of results from in vitro antimicrobial susceptibility testing, especially for certain veterinary bacterial pathogens. The veterinary diagnostic laboratory serves a vital role in relaying accurate antimicrobial susceptibility information to practicing clinicians and may be an active participant in monitoring and surveillance programs, especially those involved in the drug approval process. The fulfillment of these responsibilities necessitates the use of standardized in vitro antimicrobial susceptibility testing methods that have been approved by a national or international organization.

The importance of antimicrobial agents in the treatment of infectious diseases of bacterial etiology has been recognized for over 50 years. However, how to select the appropriate antimicrobial agent for treatment is not as well established. Several factors should be taken into consideration when choosing an antimicrobial agent for clinical use. These include the nature of the infection, the identity and susceptibility of the pathogen, the pharmacokinetic behavior of the chosen drug in the target animal species, the pharmacodynamic indices of the drug at the site of infection, host characteristics (e.g., site and nature of the infection and toxicity to the host), the cost, the ease, route and frequency of administration and, in food animals, the residue avoidance time.

Although the majority of these factors may be elucidated in the drug development and approval process, the susceptibility of the pathogen and the pharmacodynamic indices at the site of infection may be unique to each animal and pathogen interaction. The pharmacodynamic indices that characterize the bacterial pathogen-host-antimicrobial agent interaction are discussed in Chapter 5.

The chapter presented here addresses issues relative to determining the susceptibility of a bacterial pathogen to an antimicrobial agent. This will include the various types and components of the different antimicrobial susceptibility testing (AST) methods, how each may affect the results and how results are reported.

Traditionally, the results from in vitro antimicrobial tests are reported qualitatively and/or quantitatively. Qualitative results are reported as susceptible, intermediate or resistant, whereas quantitative results are reported as minimal inhibitory concentration (MIC) in $\mu\text{g/ml}$ or mg/L . The basis for determining the qualitative interpretation will also be discussed.

The target of antimicrobial chemotherapy is the bacterium causing the infectious disease process. Antimicrobial agents that are marketed for clinical use are selected from a plethora of compounds due, in part, to their selective toxicity for bacterial pathogens and their negligible effect on animal tissues and organs. However, not all bacterial pathogens, even those of the same species, are equally susceptible to an antimicrobial agent. For example, the susceptibility of a pathogen such as *Pasteurella multocida* may vary greatly to the same antimicrobial agent (Table 2.1) (Kehrenberg et al., 2006). In this example, the suscep-

Table 2.1. In vitro susceptibility data of *Pasteurella multocida* isolates from different animal sources.

Source	No. of Isolates	MIC Data (μg/ml)	Ampicillin	Gentamicin
Swine/USA	715	MIC ₅₀	0.25	2
		MIC ₉₀	0.25	4
		Range	≤0.06 – ≥64	≤0.12 – ≥16
Swine/Germany	442	MIC ₅₀	<0.12	1
		MIC ₉₀	0.5	2
		Range	≤0.12 – ≥256	≤0.06 – 64
Cattle/Germany	132	MIC ₅₀	<0.12	0.5
		MIC ₉₀	1	2
		Range	≤0.12 – ≥256	≤0.06 – ≥128
Cattle/Germany	154	MIC ₅₀	0.12	2
		MIC ₉₀	0.25	4
		Range	≤0.03 – ≥32	≤0.03 – 8
Dogs, Cats/USA	112	MIC ₅₀	0.12	1
		MIC ₉₀	0.25	2
		Range	≤0.03 – 0.5	≤0.12 – 4

tibility of *P. multocida* isolates from various sources to two antimicrobial agents of totally different classes varies considerably. The susceptibility of *P. multocida* to ampicillin ranged from ≤ 0.03 to ≥ 256 μg/ml among various animal sources and countries of origin. Even isolates within the same country exhibited considerable variation. Similar variations in susceptibility can be seen with gentamicin. Although the isolates shown in Table 2.1 are from different animal species and different geographic locations, variation in susceptibility may be present in bacteria of the same species isolated from a single specimen (Figure 2.1). In addition, the susceptibility of a population of bacteria can change over time, especially if there is repeated or continual exposure to antimicrobial agents. The variability in susceptibility of bacterial pathogens to the various antimicrobial agents available for clinical use can be accurately assessed by subjecting the bacterial pathogens to properly conducted in vitro antimicrobial susceptibility tests. Results from such tests can provide guidance as to which of the numerous antimicrobial agents might be appropriate to use in this era of ever changing susceptibility profiles. The importance of the use of properly conducted in vitro antimicrobial tests lies in the fact that essentially all decisions regarding the treatment of an infectious disease

process with an antimicrobial agent are based on the premise that in vitro antimicrobial susceptibility tests are predictive of in vivo therapeutic efficacy. However, the ability of an in vitro test to predict the clinical effectiveness of an antimicrobial agent is dependent on that test being performed correctly. Tests that are performed using non-standardized testing methods are one source of doubt as to the effectiveness of in vitro testing in that they most likely will generate erroneous results that could contribute to therapeutic failures, as will be discussed below.

The first in vitro antimicrobial susceptibility tests were developed shortly after the discovery of the inhibitory effects of penicillin on *Staphylococcus aureus* (Fleming, 1929). Both broth dilution and disk diffusion tests were employed in early studies. However, a majority of these tests were developed without regard for intra- or inter-laboratory reproducibility, and were usually performed by laboratory workers who lacked scientific or technical training (Ambrose, 2005). For the most part, laboratories simply developed a method that fit their needs, resulting in the development of a variety of testing procedures. With the proliferation of testing procedures, it became increasingly difficult to compare results, not only among communities but even within the same hospital over time. To remedy

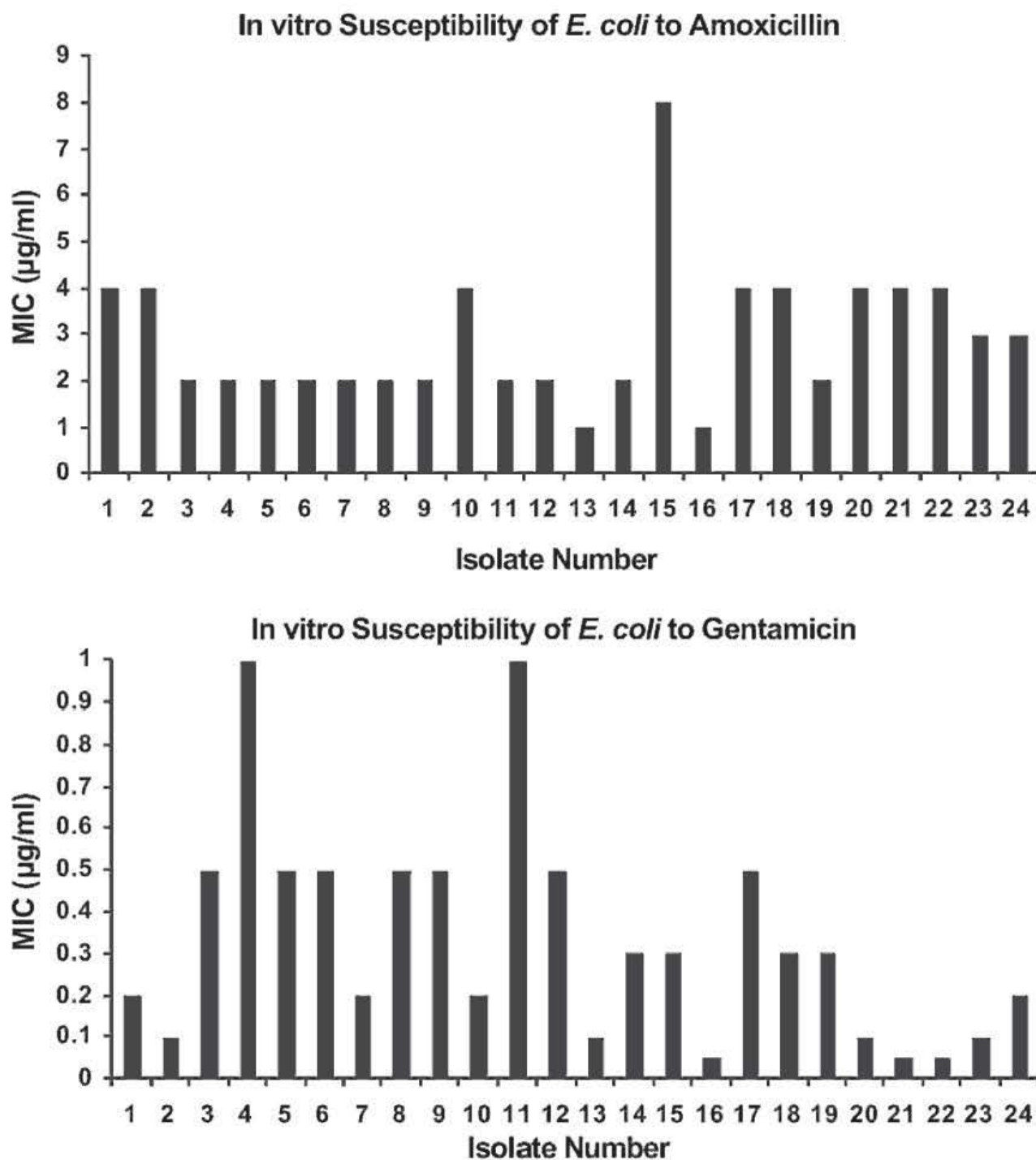


Figure 2.1. In vitro susceptibility of *E. coli* isolates from a single sample to amoxicillin and gentamicin.

this situation, the World Health Organization put forth recommendations for the development of standardized antimicrobial susceptibility testing methods. In the ensuing years, confusion associated with AST attracted the attention of both the judicial and legisla-

tive branches of the U.S. Federal government. This was followed shortly thereafter by the adoption of a disk diffusion method described by Bauer et al. (1966). This testing method gained widespread acceptance and became the basis for disk diffusion standards

adopted by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards or NCCLS).

Indications for In Vitro Antimicrobial Susceptibility Testing

Although there may be considerable variation in the susceptibility of bacterial pathogens to antimicrobial agents, there are still some bacteria that are uniformly susceptible to inexpensive, nontoxic agents so that, under most circumstances, these bacteria do not need to be tested. Examples include the β -hemolytic streptococci and *Arcanobacterium pyogenes*, which are universally susceptible to penicillin in vitro and in vivo. Susceptibility testing of these organisms may provide a practitioner with misleading information, e.g., indication of a susceptibility phenotype which would not be affected by a particular drug in vivo or would suggest the possible use of more expensive or toxic agents. On the other hand, some bacteria are intrinsically resistant to particular antimicrobial agents, and it is therefore inappropriate and potentially misleading to test these bacterial pathogen/antimicrobial agent combinations. Examples include *Escherichia coli* and vancomycin, *Salmonella* and penicillin G, and enterococci and cephalosporins. In addition, bacteria that are typically considered to be contaminants or normal flora should not be tested. The reasons these organisms should not be tested include the expense associated with unnecessary testing, the possibility that such testing will result in the clinician's treating normal flora, the potential of a clinician selecting a more expensive or more toxic agent, and the risk of selecting for resistant organisms through inappropriate use of antimicrobial agents.

On the other hand, in vitro testing should be done when the susceptibility of the pathogen cannot be predicted based on the clinician's experience, the identity of the pathogen is needed, or as a guide in choosing the most appropriate antimicrobial agent to use in a clinic, hospital, or community setting. Although in vitro AST results have been used successfully to predict clinical responses, they reflect static tests performed under carefully standardized conditions. In the in vivo environment from which the pathogen was isolated, nothing is standardized or static (including pH, oxygen tension, bacterial concentration, or the ability of

the drug to diffuse into or away from the site of the infection). Thus, the results generated from the in vitro testing may not always be applicable to the in vivo use of an antimicrobial agent due to a variety of factors associated with the antimicrobial agent, the host or the pathogen.

Specifically, some antimicrobial agents perform very poorly under the circumstances in which they are used, even though the in vitro results indicated that the bacterium would be susceptible. For example, *E. coli* isolated from an anaerobic infection and shown to be susceptible to gentamicin in vitro will not respond to this drug in vivo for a number of reasons, including the lack of an oxidative transport system to carry the drug across the bacterial membrane, the poor diffusion of the drug into the site of the infection, and inactivation of the drug by purulent exudate. The presence of a bacterial biofilm will also impede the effectiveness of an antimicrobial agent. Biofilms form when bacteria are allowed to colonize viable and nonviable tissue or material within the host. Foreign bodies or sequestra provide ideal surfaces for such colonization. Once embedded in these biofilms, the bacteria are, for the most part, out of reach of antimicrobial therapy. These and other factors causing disparate in vitro and in vivo results are detailed in Chapters 4 and 5.

Despite these differences, when tested under standardized conditions, the use of antimicrobial agents to which the tested pathogens are susceptible in vitro has a high probability of success, whereas the use of antimicrobial agents to which they are resistant predicts a poor clinical response (Craig, 1993). One of the major reasons for this is the quality of data used by standards-setting organizations to generate the respective antimicrobial/bacterium interpretive criteria.

Susceptibility Testing Methods

A variety of methods have been developed for in vitro antimicrobial susceptibility testing of bacteria from clinical specimens. As indicated above, in vitro antimicrobial susceptibility testing is sometimes performed by individuals who have very little knowledge, training, or experience in microbiology. The potential variability in testing methods and technical skills dictate that all in vitro antimicrobial susceptibility test results should be generated by strict adherence to

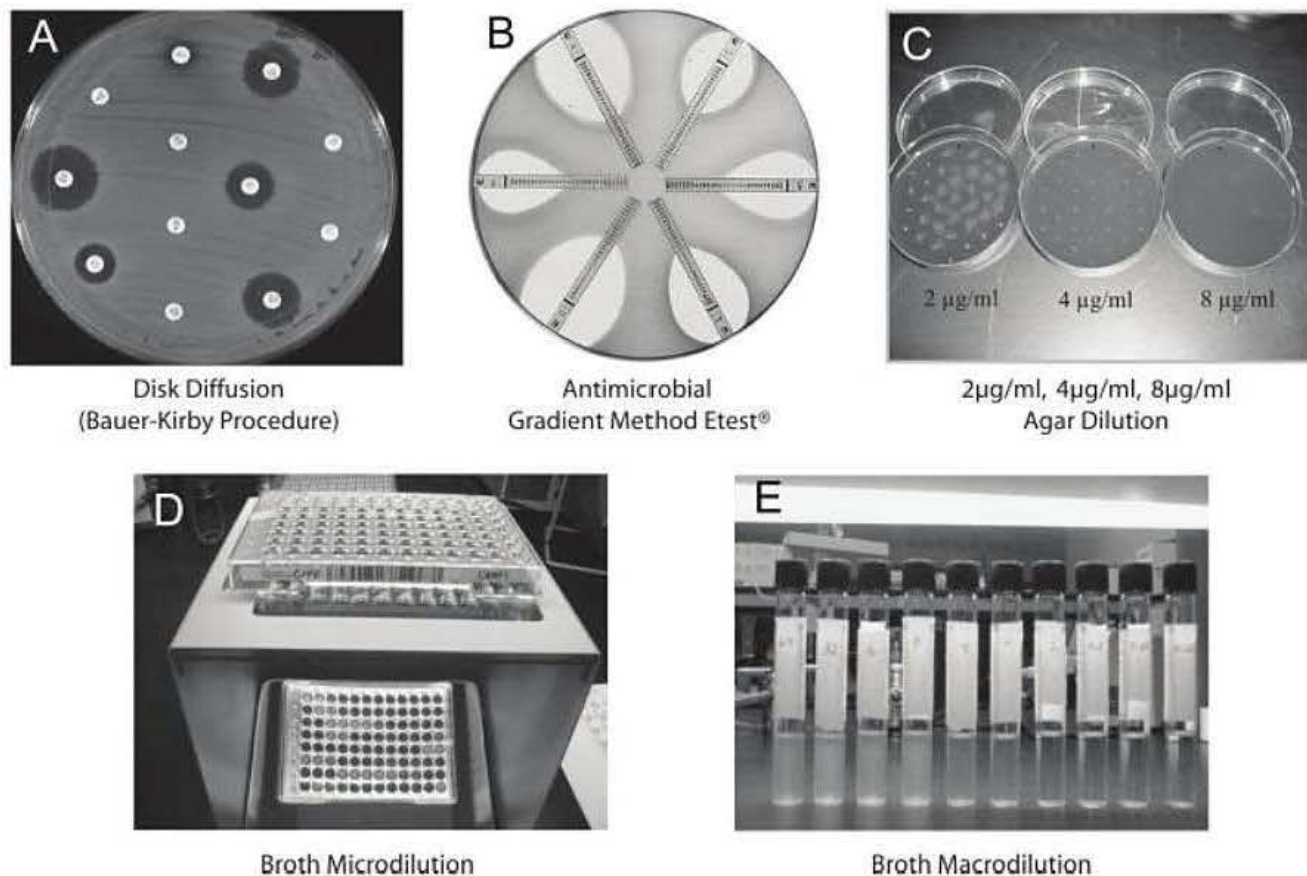


Figure 2.2. Antimicrobial susceptibility testing methods.

standardized methods such as those described by CLSI. Although there are a number of testing methods, most tests are performed on aerobic and facultative anaerobic bacteria and with antimicrobial agents that are intended for systemic use. These methods use inhibition of the bacterium, rather than killing, as the end point.

In vitro susceptibility tests are limited to two basic procedures. These are diffusion, disk or concentration gradient; and dilution, agar or broth (microdilution or macrodilution) (Figure 2.2). The disk diffusion and the broth microdilution tests are the ones most commonly used in veterinary medicine. The Etest®, a concentration gradient testing method, has also been used for a wide variety of microbial pathogen/antimicrobial agent combinations. The decision on which test method to use is based on cost, ease of use, and flexibility as it relates to the capabilities of the laboratory personnel and the needs of the clientele.

Disk Diffusion Test

The disk diffusion test is the testing method most widely used in veterinary medicine because of its flexibility in type and number of drugs that can be tested on a daily basis and its relatively low cost. As the name implies, the disk diffusion test is based on the diffusion of an antimicrobial agent from a disk (usually commercially prepared and thus standardized, if stored properly) placed on an agar surface of standardized growth medium (e.g., Mueller-Hinton agar) that has been seeded with approximately 1 to 2×10^8 colony-forming units (CFU)/ml of a pure culture of the test bacterium. When the disk is applied to the seeded agar surface, a “race” occurs between the growth of the bacterium on the agar surface and the diffusion of the drug through the agar. The diffusion of the antimicrobial agent results in a drug concentration gradient (Figure 2.3). When the concentration of the antimicrobial agent becomes too dilute to inhibit the growth



Figure 2.3. Disk Diffusion. The diffusion of an antimicrobial agent from a defined reservoir through a solid medium seeded with a defined concentration of a bacterium. A zone of inhibition forms when the concentration of the drug becomes too dilute to inhibit the growth of the bacterium.

of the bacterium, a zone of inhibition is formed (Figure 2.4a). In general, this zone of inhibition correlates inversely with the MIC of the test organism. In other words, the larger the zone of inhibition, the smaller the concentration of drug required to inhibit the pathogen.

Several factors can adversely affect the outcome of this test. For example, if the agar is too thin, e.g., less than the recommended 4 mm, there may be an increase in the lateral diffusion of the antimicrobial agents resulting in increased zone sizes (Figure 2.4b). On the other hand, if the agar is thicker than the recommended 4 mm, there may be a decrease in lateral diffusion which may result in decreased zones of inhibition (Figure 2.4c). If the concentration of bacteria used to inoculate the surface of the agar is less than the recommended 1 to 2×10^8 CFU/ml, as is usually the case with plates inoculated directly from clinical specimens, or if the pathogen being tested has a long generation time (slow grower, e.g., *A. pyogenes*) the zones of inhibition will be larger than they should be (Figure 2.4d). This is in contrast to zones of inhibition that are smaller than they should be if there were too many bacteria in the original inoculum (Figure 2.4e). Finally, if the plate inoculum is a mixed culture, which is frequently the case in plates inoculated directly from a clinical sample, the organisms will be in competition for the available nutrients. This may result in erroneous zones of inhibition for all isolates on the plate, but especially the pathogen if the commensal has a faster generation time (Figure 2.4f). Zones of inhibition that are larger than they should be, due to testing error, may make some bacteria appear susceptible that might be resistant if tested under the appropriate standardized testing conditions. The reverse is true when smaller zones of inhibition are generated from inappropriate testing procedures. Another factor that can affect the results of the disk diffusion test is the place-

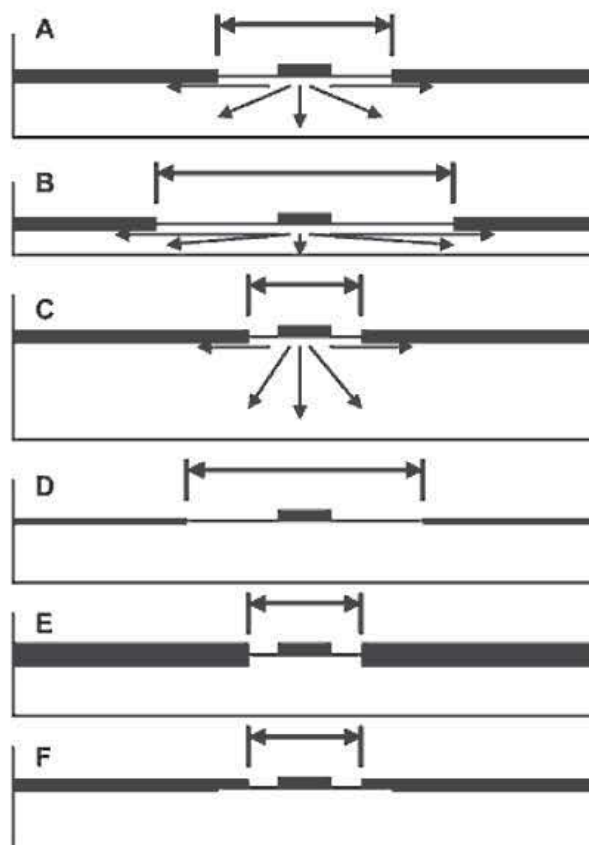


Figure 2.4. Disk Diffusion—The results of the disk diffusion test can be influenced by the depth of the medium (a; b, increase in zone of inhibition; c, decrease in zone of inhibition) or the quality of the inoculum (d, false increase in zone of inhibition; e, false decrease in zone of inhibition; f, mixed culture, false decrease in zone of inhibition).

ment of the disks. CLSI requires that the disks are evenly distributed so that the zones of inhibition do not overlap. This can be accomplished by placing the disks 24 mm apart, center to center. To ensure the test is performed properly, laboratories should adhere to the criteria listed in the standards documents from which the interpretive criteria are obtained.

The major disadvantage of the disk diffusion test is that results are qualitative, e.g., reported as susceptible, intermediate or resistant (SIR). Results such as this can sometimes put the clinician at a disadvantage in treating an infectious disease process. By knowing how susceptible a bacterial pathogen is to an antimicrobial agent, the clinician has the option in some instances of adjusting the dose to reduce cost, reduce potential toxicity, or increase bacterial killing. The value of gener-

ating quantitative information for the clinician has prompted the development of two variations of the disk diffusion test. The first is a computerized system that calculates the MIC of an antimicrobial agent from the zone of inhibition generated by the disk diffusion test and the second is a concentration gradient system. Both systems were developed for use in human medicine but have seen application in veterinary medicine. The interpretation of the data generated by the computerized system is based on data collected from testing bacterial isolates from humans. This system has been tested on bacteria isolated from animals and found to be potentially useful to the veterinary community, but would require the development of a database that was more specific to veterinary medicine in order to include many important animal-bacterial pathogen combinations (Hubert et al., 1998).

The concentration gradient strip (Etest®, Epsilon-meter) is a modification of the diffusion test that generates quantitative results. The test relies on the diffusion of a continuous concentration gradient of an antimicrobial agent from a plastic strip into an agar medium seeded with a pure culture of the test bacterium. The plastic strip has a defined concentration of a stabilized dried drug on one side, in a continuous gradient from top to bottom, and a continuous MIC interpretive scale on the reverse side. After incubation, the MIC is determined by reading the concentration on the strip where the zone of inhibition intersects the strip. Because the Etest uses a concentration gradient, MIC values between the standard two-fold dilution can be obtained. In performing this test, special attention must be paid to carefully placing the strips on the agar surface. In addition, users should be aware that there may be discrepancies between MICs generated with the Etest and those generated by traditional dilution methods for some bacterium/antimicrobial agent combinations (Brown and Brown, 1991; Ge et al., 2002). The high cost of the Etest inhibits its use for routine testing in the veterinary clinical bacteriology laboratory, but it is useful under those circumstances where AST results are needed for specific drug/microbial pathogen combinations.

Dilution Susceptibility Tests

Quantitative susceptibility testing may be performed using agar dilution, broth macrodilution, or broth microdilution. These testing methods traditionally involve serial two-fold dilutions indexed to the log base

2 (e.g., 0.5, 1, 2, 4, 8, 16 µg/ml, etc). Of these, agar dilution has been referred to as the “gold standard”. However, both agar dilution and broth macrodilution are generally too cumbersome for routine clinical use. The broth microdilution test, on the other hand, is being used with increasing frequency in veterinary laboratories and in surveillance programs. This test is performed in microtiter plates, with round or truncated V-bottom wells, using antimicrobial agents of known potency in progressive two-fold dilutions that encompass drug concentrations similar to those obtained in serum and tissue following administration at U.S. Food and Drug Administration–approved or standard-of-practice doses (CLSI 2002a).

The microtiter trays may be prepared using a variety of formats, but each tray usually contains several antimicrobial agents that are tested against a single isolate. The microtiter trays may be prepared in-house or obtained commercially. Those obtained commercially may be purchased as dehydrated or frozen trays (CLSI reference method). Dehydrated trays generally have a shelf life of one to two years and may be stored at room temperature, whereas frozen trays have a shelf-life of six months and must be stored at -10°C or -70°C, depending on the antibacterial agents contained in the tray. Microdilution tests are more expensive than the disk diffusion test and lack the day-to-day flexibility, in terms of choice of antimicrobial agents to test, seen with the disk diffusion test. This problem of lack of flexibility may be compounded by bulk buying (to reduce cost) and the need to finish using a two-year supply of plates before introducing a new antibiotic to the panel. To reduce the cost associated with microtiter dilution trays some laboratories use “breakpoint” trays. These have more antimicrobial agents than the “full range” trays, but each agent has only two or three dilutions, usually one dilution below the susceptible breakpoint, the susceptible breakpoint, and sometimes one dilution above the susceptible breakpoint. The advantage of this is that susceptibility testing can be automated; the disadvantage is that it provides little more information than disk diffusion testing.

Another modification of the breakpoint panel is to use fewer antimicrobial agents but design the panel so that two or three organisms may be tested on it. Still a third modification of the broth microdilution method is the use of automated susceptibility testing methods. These systems rely on increases in bacterial growth in

Drug	Concentration ($\mu\text{g}/\text{ml}$)												MIC
	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	
A	●	●	●	●	●	●	○	○	○	○	○	○	2
B	●	●	●	●	●	○	●	○	○	○	○	○	Repeat (?)
C	●	●	○	○	●	●	○	○	○	○	○	○	Repeat
D	●	●	●	●	●	●	●	●	○	○	○	○	8
E	○	○	○	○	○	○	○	○	○	○	○	○	≤ 0.03
F	●	●	●	●	●	●	●	●	●	●	●	●	>64
G	●	●	●	●	●	●	●	●	○	○	○	○	Repeat
H	●	○	○	○	○	○	○	○	○	○	○	○	0.06
Controls													

Figure 2.5. Broth Microdilution. Results are determined by the growth of the bacterium in the presence of varying concentrations of the test drug. The MIC is usually read as the first well with no obvious growth.

positive control wells. When the appropriate turbidity is reached, the turbidity in each well is read and the susceptibility of the isolate determined by using an algorithm for that drug. Like the “breakpoint” panel, this testing method does not generate full-range MIC data. Because of the limited dilution range used in breakpoint panels and automated systems, the results generated can only be reported qualitatively and are therefore similar to but often far more expensive than those generated by a disk diffusion test.

To perform a broth microdilution test, a bacterial suspension is made from an overnight culture or a culture in a logarithmic phase of growth, and diluted to a turbidity comparable to that of a 0.5 McFarland turbidity standard (approximately 1 to 2×10^8 CFU/ml). This suspension is further diluted in sterile water, saline, or broth so that ultimately the final concentration of bacteria per well is approximately 5×10^4 CFU. The suspension is then used to inoculate a microtiter tray that contains serial two-fold dilutions of the antimicrobial agents to be tested. Once inoculated, the trays are stacked two to four trays high in a 35°C in-

cubator with ambient air and incubated for 16 to 20 hours.

When reading the microtiter trays, the MIC is recorded as the lowest concentration of antimicrobial agent that completely inhibits the growth of the organism, as determined by the unaided eye. For example, in Figure 2.5, for Row A, the MIC would be read as $2 \mu\text{g}/\text{ml}$. For Row B the MIC could be read as $4 \mu\text{g}/\text{ml}$ (CLSI’s recommendation) or the test could be repeated. Experience in our laboratory suggests that the MIC will be $1 \mu\text{g}/\text{ml}$ and thus the test should be repeated. The test conducted in Row C should also be repeated because of the two skipped wells. The MIC for Row D is $8 \mu\text{g}/\text{ml}$ and $\leq 0.03 \mu\text{g}/\text{ml}$ for Row E. For Row F the MIC is >64 . Row G should be repeated as it appears to be a mixed culture when one compares it to the positive control wells. One should keep in mind that the MIC is not an absolute value. The true MIC may be a concentration of drug that is between the last concentration where there was visible growth and the first concentration where there was no growth. For example, if there was growth at $4 \mu\text{g}/\text{ml}$ but no growth at

8 µg/ml the MIC would be recorded as 8 µg/ml when in fact the actual MIC may be somewhere between 4 and 8 µg/ml. Obviously, the greater the dilution, e.g., 0.008 µg/ml, the less relevant this issue becomes. It should also be noted that when performing dilution testing, the dilution range for each drug should encompass the established interpretive criteria and also the quality control (QC) range of at least one of the QC organisms to be used. To encompass the QC range the dilution range must be at least one dilution below the end of the QC range, e.g., if the QC range is 0.06 to 0.25 µg/ml the test dilution range should begin at 0.03 µg/ml.

Determining Interpretive Criteria

The results generated by *in vitro* antimicrobial susceptibility tests, whether they are determined by diffusion or dilution methodologies, are generally provided to clinicians by designating the tested pathogen as susceptible, intermediate, or resistant to the various antimicrobial agents it was tested against. These designations are reached by determining *in vitro* breakpoints – those zones of inhibition or MICs at which an organism is considered to be susceptible, intermediate, or resistant based on obtainable serum concentrations of the drug and the results of clinical trials. In other words, when a laboratory reports that an organism is susceptible to a particular antimicrobial agent, it implies that when administered to the target animal species at the FDA (or appropriate government agency) approved route and dose, the serum or tissue concentrations of the antimicrobial agent will be sufficient to inhibit the bacterium's growth *in vivo*. The resistant breakpoints, on the other hand, are for those isolates that are not inhibited by usually achievable concentrations of a drug following normal administration schedules; thus, clinical efficacy of the drug is unreliable. Intermediate breakpoints are those zones of inhibition or MICs that fall between the susceptible and resistant breakpoints. Traditionally these have represented a “buffer zone” that prevented resistant organisms from being categorized as susceptible, or vice versa, or to compensate for uncontrolled technical problems in the laboratory. However, this category may also be used to treat a bacterium at a body site where the drug may be concentrated, e.g., amoxicillin in urine, or when a higher dosing regimen can be used because of the drug's wide pharmacotoxicity margins.

Antimicrobial susceptibility test breakpoints are necessary for the correct clinical interpretation of quantitative or qualitative susceptibility test values of antibacterial agents. SIR breakpoints are carefully selected by regulatory agencies or professional organizations (e.g., CLSI) based on an extensive review of the microbiological, pharmacokinetic, pharmacodynamic, and clinical data available for each bacterium/antimicrobial combination. Throughout the world, different governmental agencies and professional organizations have responsibility for the initial establishment of antimicrobial susceptibility breakpoints. In the United States, CLSI has a mechanism in place to initially establish breakpoints and publish updates on an annual basis. This process also includes periodic reevaluation of breakpoints as changes in susceptibility testing methods, antimicrobial formulations, or resistance phenotypes occur (Ferraro, 2001)

Interpretive criteria used in veterinary medicine may come from one of three sources. The first is from a drug's sponsor. In this scenario the sponsor of a new antimicrobial agent, once a drug has been approved for use, provides information to the testing laboratories relative to how the laboratory should interpret the *in vitro* test results. They may or may not include QC recommendations. The second source is from interpretive criteria set by CLSI for antimicrobial drugs used in humans. These interpretive criteria are based on information submitted by the drug's sponsor on bacterial population dynamics using bacterial isolates from humans, pharmacokinetic data generated in humans, and clinical trials conducted in humans. However, the validity of these types of data has not been established for many veterinary pathogen/antimicrobial agent combinations. The third source is from a standards-setting organization such as the CLSI's Subcommittee on Veterinary Antimicrobial Susceptibility Testing, which evaluates data that is specific for the intended drug/bacterial pathogen combination in the targeted animal species.

Determining when to interpret a bacterium as susceptible or resistant to an antimicrobial drug involves two phases in the CLSI process. The first phase is to establish testing methods and the QC ranges for the drug/QC organism combinations. If appropriate, both the diffusion and dilution testing methods are used. The purpose of this is to validate the *in vitro* testing of the clinical isolates the antimicrobial agents will be

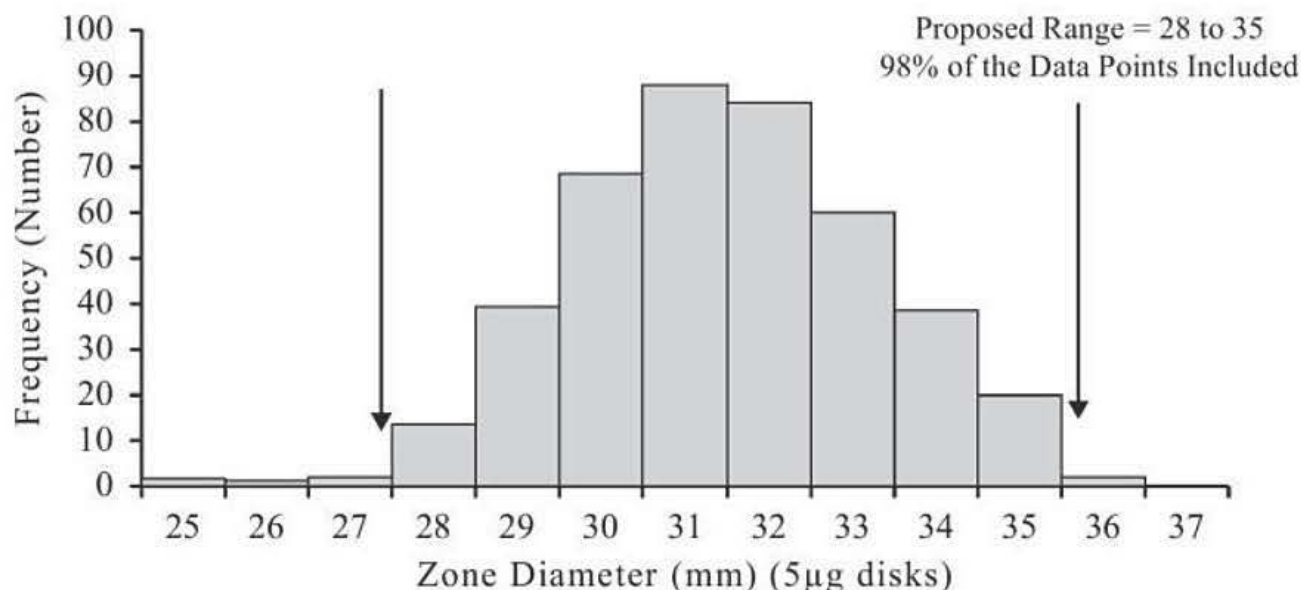


Figure 2.6. Example of zone diameter data points generated in a seven-laboratory study designed to define the quality control limits of a new antibacterial agent against a standardized quality control bacterium.

tested against. The second phase involves the determination of the interpretive criteria.

To evaluate the performance of in vitro susceptibility tests, the limits of acceptable variability in zones of inhibition or MIC ranges are first defined by testing the agent against all appropriate QC strains of bacteria. These strains are from a single source, usually the American Type Culture Collection. In the early part of this phase of development, the agent is tested against the QC organisms at different pHs and at different concentrations of bacteria to determine the influence these variables have on the test results. Broth microdilution test results are also compared with agar dilution test results. Once these tests have been performed, the agent is tested in a minimum of seven different laboratories, usually using both the disk diffusion and the broth microdilution testing methods using a minimum of three different lots of media. For the disk diffusion test, two lots of disks are evaluated. At least 10 separate tests are performed for each QC organism over 10 days. A control drug of the same class as the test agent, that has already had QC guidelines established, is run concurrently with the test agent. Once the QC ranges for the QC organisms have been established (Figs. 2.6 and 2.7), the interpretive criteria can be determined.

The determination of the interpretive criteria in-

volves generating three pieces of data: (1) the in vitro susceptibility of the bacterial species for which the test agent is being marketed, generated under standardized testing conditions (population distribution); (2) the pharmacokinetic parameters of the test agent administered at the approved dose(s) in the target animal species; and (3) the results of clinical trials. Ideally, the population distribution study is performed on 300 to 600 (50 to 100 isolates may be used if a single bacterial species is the target of the therapy) recent clinical isolates representing all species of bacteria likely to be tested against the antimicrobial agent, using both the disk diffusion and broth microdilution testing methods. These isolates should represent a wide geographic distribution but may be determined in a single laboratory in accordance with the CLSI M37-A2 document (CLSI, 2006b). Interpretation of the data involves generating a scattergram by plotting the zone of inhibition against the MIC for each pathogen and calculating a linear regression line (Figure 2.8). The selection of potential breakpoints—susceptible, intermediate, and resistant—is then based on regression line analysis, error rate-bounding. These breakpoints are then adjusted, if necessary, by analysis of pharmacokinetics data in the target animal species and the results of properly conducted clinical trials that measure clinical and bacteriological response rates.

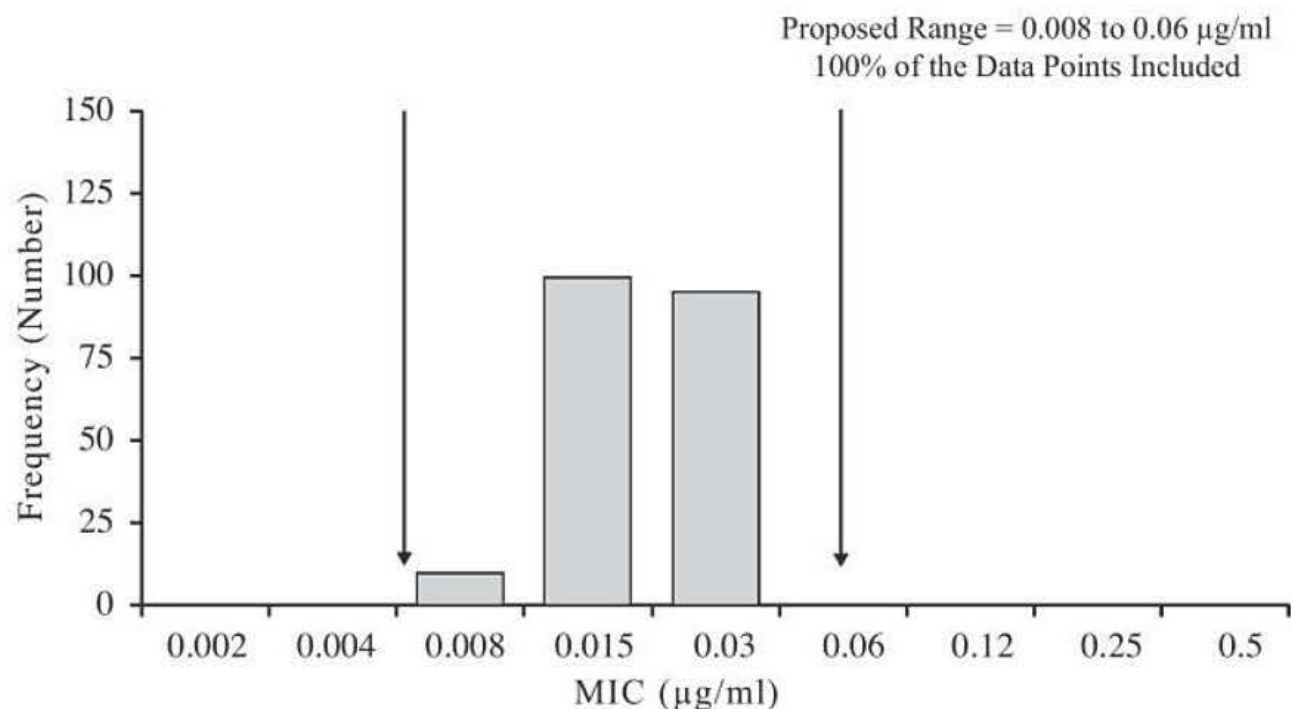


Figure 2.7. Example of MIC data points generated in a seven-laboratory study designed to define the quality control limits of a new antibacterial agent against a standardized quality control bacterium.

Interpretation of In Vitro Antimicrobial Susceptibility Test Results

A variety of reasons exist to suspect that in vitro tests cannot always predict the efficacy of an antimicrobial agent in vivo. In vitro tests involve the continuous exposure of a relatively small concentration of bacteria to a constant level of an antimicrobial agent under standardized testing conditions. These conditions differ considerably from the in vivo conditions from which the bacterial pathogen was isolated. For example, in a disease entity such as bovine pneumonia the pathogen may be present in much larger numbers than tested in vitro. In addition the pathogen is exposed to fluctuating levels of the drug at potentially wide variations in pH and oxygen tension, and it must evade host defenses. Despite these considerable differences, studies in human medicine have demonstrated the clinical value of in vitro susceptibility tests (Gudmundsson and Craig, 1986; McCabe and Treadwell, 1986; Lorian and Burns, 1990; Stratton, 1991; Craig, 1993; Johnson, 1996; Ambrose, 2005). Thus, when antimicrobial chemotherapy fails there are a number of possible reasons. Traditionally, the most obvious rea-

son has been because the test results were inaccurate. This is most likely true if the test was not performed correctly. On the other hand, if the test was performed under standardized testing conditions and the QC data were within range, the results should be considered to be correct. The therapeutic failure could be due to the selection of the wrong agent, e.g., an aminoglycoside to treat an isolate from a purulent exudate; the wrong dosing regimen, e.g., low dose of a fluoroquinolone to treat a bacterial pathogen with a high, but still susceptible MIC; or incorrect frequency of dosing, e.g., use of a penicillin with a short elimination half-life that is administered every twelve hours; or the host defenses or immune system of the host has been severely compromised. Failure can also result if the infection is a heavy concentration of bacteria walled off in an abscess, or if bacteria are intracellular, or if there is extensive necrosis preventing access of the antimicrobial drug to the site of infection. The selection of the appropriate dose can be driven by the result of quantitative susceptibility tests, as will be discussed in Chapter 5. For example, studies have shown that bacterial pathogen/antimicrobial agent combinations that

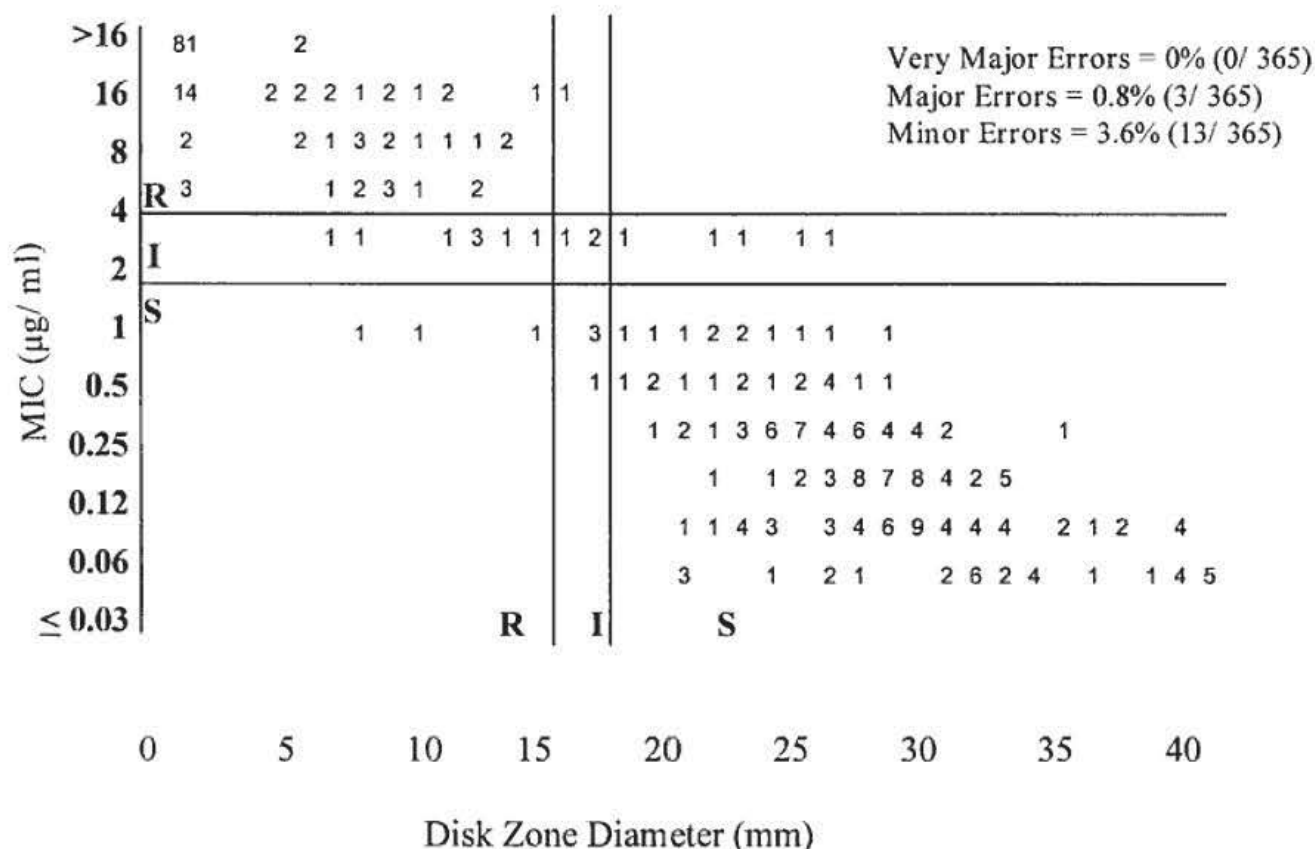


Figure 2.8. Scattergram comparing MICs and zones of inhibition which, in conjunction with pharmacokinetic data and results from clinical trials, contribute to the development of interpretive criteria. Numbers refer to number of isolates at each point. R, resistant; I, intermediate; S, susceptible.

produce small zones of inhibition, though still considered to be susceptible, have been associated with higher rates of bacteriological treatment failure than bug/drug combinations that produce larger zones of inhibition (Gerber and Craig, 1981; Thornsberry et al., 1982). The same types of results have been seen with patients treated with antimicrobial agents that had high, but susceptible, MICs (small zones of inhibition) against the pathogens (Craig, 1993).

By generating full-range MICs, a laboratory can give clinicians information that may allow them to individualize the therapeutic regimen, especially with regard to dose and dosing frequency. For example, if the MIC is very low, the dose or frequency of dosing may be decreased. On the other hand, if the MIC is higher, but the organism is still considered susceptible and the drug has a wide pharmacotoxicity margin, a higher dose of the drug may be used. In other words, interpretation of susceptibility testing depends on knowing

the relationship between in vitro susceptibility and factors involved in relation to tissue drug concentrations (which depend on factors such as dose and pharmacokinetic and pharmacodynamic properties of the drug or drug class, discussed in Chapter 5). As costs associated with antimicrobial therapeutic use continue to increase and reports of decreased susceptibility of many bacteria to commonly used antimicrobial agents grow, standardized AST and its appropriate interpretation will lead to a greater chance of using these drugs more successfully.

Additional Antimicrobial Susceptibility Testing Applications

In special circumstances, novel test methods and assays may be more appropriate for detection of particular resistance phenotypes. For example, chromogenic

cephalosporin-based tests (CLSI 2002a) (e.g., nitrocefin) or equivalent methods may provide more reliable and rapid results for β -lactamase determination in certain bacteria (CLSI 2002a). Similarly, extended-spectrum β -lactamase (ESBL) (CLSI 2002a) activity in certain bacteria can also be detected by using standard disk diffusion susceptibility test methods using specific cephalosporins (e.g. cefotaxime and ceftazidime) in combination with a β -lactamase inhibitor (clavulanic acid) and measuring the resulting zones of inhibition (CLSI 2002a). Inducible clindamycin resistance in *Staphylococcus* spp. may be detected using a disk diffusion method employing standard erythromycin and clindamycin disks in adjacent positions and measuring the resultant zones of inhibition (e.g., D-zone) (Zelazny et al., 2005). Chloramphenicol resistance attributed to production of chloramphenicol acetyl transferase can be detected in some bacteria via rapid tube or filter paper tests within one to two hours (CLSI 2002a).

Future Directions in Antimicrobial Susceptibility/Resistance Detection

Antimicrobial susceptibility testing among clinical laboratories has become more complex over the years with the introduction of new methods and standards. In the near future veterinary microbiologists must also become familiar with certain antimicrobial resistance mechanisms and what methods may be used to accurately detect them. For example, the use of genotypic approaches for detection of antimicrobial resistance genes has been promoted as a way to increase the rapidity and accuracy of susceptibility testing (Cai et al., 2003). Numerous DNA-based assays are being developed to detect bacterial antimicrobial resistance at the genetic level. The newest and perhaps most state-of-the-art approach is to predict antimicrobial resistance phenotypes via identification and characterization of the known genes that encode specific resistance mechanisms.

Methods that employ the use of comparative genomics, microarrays, nucleic acid amplification techniques (e.g., polymerase chain reaction (PCR), and DNA sequencing offer the promise of increased sensitivity, specificity, and speed in the detection of known resistance genes. Genotypic methods have been successfully applied to supplement traditional AST

phenotypic methods for other organisms including methicillin-resistant staphylococci, vancomycin-resistant enterococci, and detection of fluoroquinolone resistance mutations (Cai et al., 2003; Chen et al., 2005; Perreten et al., 2005). PCR methods have been described for β -lactamases, aminoglycoside inactivating enzymes, and tetracycline efflux genes, to name a few (Chen et al., 2005; Perreten et al., 2005).

Technological innovations in DNA-based diagnostics should allow for the rapid and accurate detection of multiple resistance genes and variants in a single test. The development of rapid identification methods and genotypic resistance testing may help reduce the emergence of antimicrobial resistance by enabling the use of the most appropriate therapy from the outset. Additionally, new technological advances may facilitate the ability to probe bacterial species for large numbers of antimicrobial resistance genes quickly and cheaply, thereby providing additional relevant data to surveillance and monitoring programs.

However, it is important to remember that genotypic assays can only detect resistance, not susceptibility. Additionally, many of these molecular methods are expensive, labor-intensive, and unstandardized. Clinical studies will most likely be required to validate these genotypic approaches to detection of antimicrobial resistance (Louie, 2001). Therefore, despite the new influx of genotypic tests, standardized phenotypic AST methods will still be required to generate clinically useful information such as SIR and MICs and to detect decreases in susceptibility among bacterial pathogens.

Summation

Infectious disease processes are resolved by the elimination of invading organisms. Although antimicrobial agents may contribute to the eradication of a bacterial pathogen, it is the host's specific and nonspecific defenses that will ultimately clear the infection. The purpose of antimicrobial chemotherapy is then to assist the host's defense mechanisms in eradicating the pathogen. When antimicrobial agents are used, it is important that the appropriate drug be used at the right concentration, at the right dosing interval, and for the right length of time. Results from antimicrobial susceptibility tests, when properly performed, can assist the veterinary practitioner in choosing the correct drug, dose, and dosing regimen.

In vitro tests that are not properly performed may be poor predictors of clinical outcome. Several factors may contribute to inaccuracies in susceptibility testing. These include testing the wrong bacterium: Was it the pathogen, was it in pure culture, was it grown under the appropriate conditions, and were the correct organism and drug concentrations used? The use of appropriate QC organisms can frequently identify improper testing procedures. The interpretive criteria may also be misleading. For example, the current CLSI susceptible breakpoint for ampicillin is 8.0 µg/ml, when testing the Enterobacteriaceae and enterococci (CLSI 2002b). However, in the dog the peak serum concentration of ampicillin, when dosed at 11 mg/kg, is approximately 3.6 µg/ml. The clinical efficacy of ampicillin, as with all β-lactam antibiotics, is determined by the length of time the serum concentration exceeds the MIC of the pathogen (see Chapter 5). Thus if an organism, such as *E. coli* isolated from a soft tissue infection, has an MIC of 8.0 µg/ml when tested against ampicillin, it would be reported as susceptible even though the peak serum concentration, when dosed at 11 mg/kg, is much lower than 8.0 µg/ml. The reason for this discrepancy is that the breakpoint was not generated using data that was drug/bacterial pathogen/target animal species specific. Alternatively, dosage regimens for fluoroquinolones that produce peak serum concentrations that are only one to two times the MIC of the pathogen contribute to therapeutic failures and the selection of resistant organisms rather than to clinical and microbiological cures, even though the pathogen may have been properly identified and tested and the results indicated that it was susceptible. Errors such as this occur more frequently when qualitative results are reported without quantitative results or when the clinician does not increase the dose to treat susceptible pathogens with higher MICs.

The use of susceptibility data from properly conducted in vitro tests, in conjunction with the use of PK/PD indices, can enhance the clinical efficacy of antibacterial agents. Careful use of these two tools can reduce the potential of selecting for resistant organisms and thus prolong the usefulness of the antimicrobial agents that are currently available for clinical use. It should also be recognized that susceptibility testing is done under highly artificial circumstances which do not reflect the individual nature of the infection process; if susceptibility testing is done, it must be

done under agreed procedures. Interpretation of data depends on specific criteria, and the veterinarian should understand how interpretive criteria have been developed and where they may not apply.

Bibliography

- Ambrose P. 2005. Antimicrobial susceptibility breakpoints. PKPD and susceptibility breakpoints. *Treat Respir Med* (Suppl 4) 1:5.
- Bauer AW, et al. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Amer J Clin Path* 45:493.
- Brown DF, Brown L. 1991. Evaluation of the Etest, a novel method for quantifying antimicrobial activity. *J Antimicrob Chemother* 27:185.
- Cai HY, et al. 2003. Molecular genetic methods in the veterinary clinical bacteriology laboratory: current usage and future applications. *Anim Health Rev* 4:73.
- Chen S, et al. 2005. A DNA microarray for identification of virulence and antimicrobial resistance genes in *Salmonella* serovars and *Escherichia coli*. *Mol Cell Probes* 19:195.
- Clinical and Laboratory Standards Institute. 2002a. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; Approved Standard. CLSI Document M31-A2. Wayne, PA: CLSI.
- Clinical and Laboratory Standards Institute. 2002b. Development of in vitro susceptibility testing criteria and quality control parameters for veterinary antimicrobial agents; Approved Guidelines. CLSI Document M37-A2. Wayne, PA: CLSI.
- Craig WA. 1993. Qualitative susceptibility versus quantitative MIC tests. *Diagn Microbiol Infect Dis* 16:231.
- Ferraro MJ. 2001. Should we re-evaluate antibiotic breakpoints? *Clin Infect. Dis.* 33(Suppl 3):S227.
- Fleming A. 1929. On the antibacterial action of cultures of a penicillium with special reference to their use in the isolation of *B. influenzae*. *Brit J Exp Path* 10:226.
- Ge B, et al. 2002. Comparison of Etest and agar dilution for in vitro antimicrobial susceptibility testing of *Campylobacter*. *J Antimicrob Chemother* 50:487.
- Gerber AU, Craig WA. 1981. Worldwide clinical experience with cefoperazone. *Drugs* 22 (Suppl 1):108.
- Gudmundsson S, Craig WA. 1986. Role of antibiotics in endotoxic shock. In: Proctor RA, ed. *Clinical Aspects of Endotoxic Shock*. Amsterdam: Elsevier.
- Hubert SK, et al. 1998. Evaluation of a computerized antimicrobial susceptibility system with bacteria isolated from animals. *J Vet Diagn Invest* 10:164.
- Johnson CC. 1996. In vitro testing: correlation between bacterial susceptibility, body fluid levels, and effectiveness of antibacterial therapy. In: Lorian V, ed. *Antibiotics in Laboratory Medicine*, 4th ed. Baltimore: Williams and Wilkins.
- Kehrenberg C., et al. 2006. Antimicrobial resistance in members of the Family *Pasteurellaceae*. In Aarestrup F, ed.

- Antimicrobial Resistance for Bacteria of Animal Origin. ASM Press, Washington, D.C. 167–168.
- Louie M. 2001. Susceptibility testing. Phenotypic and genotypic tests for bacteria and mycobacteria. *Infect Dis Clin North Am.* 15:1205.
- Lorian V, Burns L. 1990. Predictive value of susceptibility tests for the outcome of antimicrobial therapy. *J Antimicrob Chemother* 25:175.
- McCabe WR, Treadwell TL. 1986. In vitro susceptibility tests: correlations between sensitivity testing and clinical outcome in infected patients. In: Lorian V, ed. *Antibiotics in Laboratory Medicine*, 2nd ed. Baltimore: Williams and Wilkins.
- Perreten V, et al. 2005. Microarray-based detection of 90 antibiotic resistance genes of gram-positive bacteria. *J Clin Microbiol* 43:2291.
- Stratton CW. 1991. In vitro testing: correlation between bacterial susceptibility, body fluid levels and effectiveness of antibacterial therapy. In: Lorian V, ed. *Antibiotics in Laboratory Medicine*, 3rd ed. Baltimore: Williams and Wilkins.
- Thornsberry C, et al. 1982. Antimicrobial susceptibility tests with cefoxime and correlation with clinical bacteriologic response. *Rev Infect Dis* 4 (Suppl):S316.
- Watts J, Yancey RJ. 1994. Identification of veterinary pathogens by use of commercial identification systems and new trends in antimicrobial susceptibility testing of veterinary pathogens. *Clin Microbiol Rev* 7:346.
- Zelazny AM, et al. 2005. Selection of strains for quality assessment of the disk induction method for detection of inducible clindamycin resistance in staphylococci: a CLSI collaborative study. *J Clin Microbiol* 43:2613.

Antimicrobial Resistance and Its Epidemiology

Patrick Boerlin and David G. White

Since the discovery of penicillin in the late 1920s, hundreds of antimicrobial agents have been developed for anti-infective therapy. Antimicrobials have become indispensable tools for decreasing morbidity and mortality associated with a host of infectious diseases and, since the introduction of antimicrobials into veterinary medicine, animal health and productivity have improved significantly (Johnston, 1998; National Research Council, 1998). However, loss of efficacy through the emergence and dissemination of bacterial antimicrobial resistance (defined as the ability of a microorganism to withstand the effect of a normally active concentration of an antimicrobial agent) is reported frequently (Davies, 1997; Kruse and Sorum, 1994; Salyers and Amiabile-Cuevas, 1997; Witte, 1998). The emergence of antimicrobial resistance was not an unexpected phenomenon. In fact, Alexander Fleming warned against the misuse of penicillin in his 1945 Nobel Prize lecture.

The frequency with which new resistant phenotypes are emerging among many bacterial pathogens in veterinary and human medicine is increasingly problematic. Infections caused by resistant bacteria are associated with higher morbidity and mortality than those caused by susceptible pathogens (Helms et al., 2002; Travers and Barza, 2002; Varma et al., 2005). In areas of concentrated use such as hospitals, this has led to lengthened hospital stays, increased health care costs, and, in extreme cases, untreatable infections. Contributing to this growing dilemma is the observation that the introduction of new classes or modifications of older classes of antimicrobials over the past six decades has been matched, slowly but surely, by the systematic development of new bacterial resistance

mechanisms. Presently, antimicrobial resistance mechanisms have been reported for all known antibiotics currently available for clinical use in human and veterinary medicine. Therefore, successful management of current antimicrobials, and the continued development of new ones, is vital to protecting animal and human health against infectious disease.

Resistance Mechanisms

A large variety of antimicrobial resistance mechanisms have been identified in bacteria, and several different mechanisms may be responsible for resistance to a single antimicrobial agent in a given bacterial species. Antimicrobial resistance mechanisms can be classified into four major categories (Figure 3.1): (1) The antimicrobial agent can be prevented from reaching its target by reducing its penetration into the bacterial cell; (2) general or specific efflux pumps may expel antimicrobial agents from the cell; (3) the antimicrobial agent can be inactivated by modification or degradation, either before or after penetrating the cell; or (4) the antimicrobial target may be modified so that the antimicrobial cannot act on it anymore, or the microorganism's acquisition or activation of an alternate pathway may render the target dispensable (Table 3.1).

Types of Antimicrobial Resistance

In the context of antimicrobial resistance, bacteria display three fundamental phenotypes: susceptibility, intrinsic resistance, or acquired resistance.

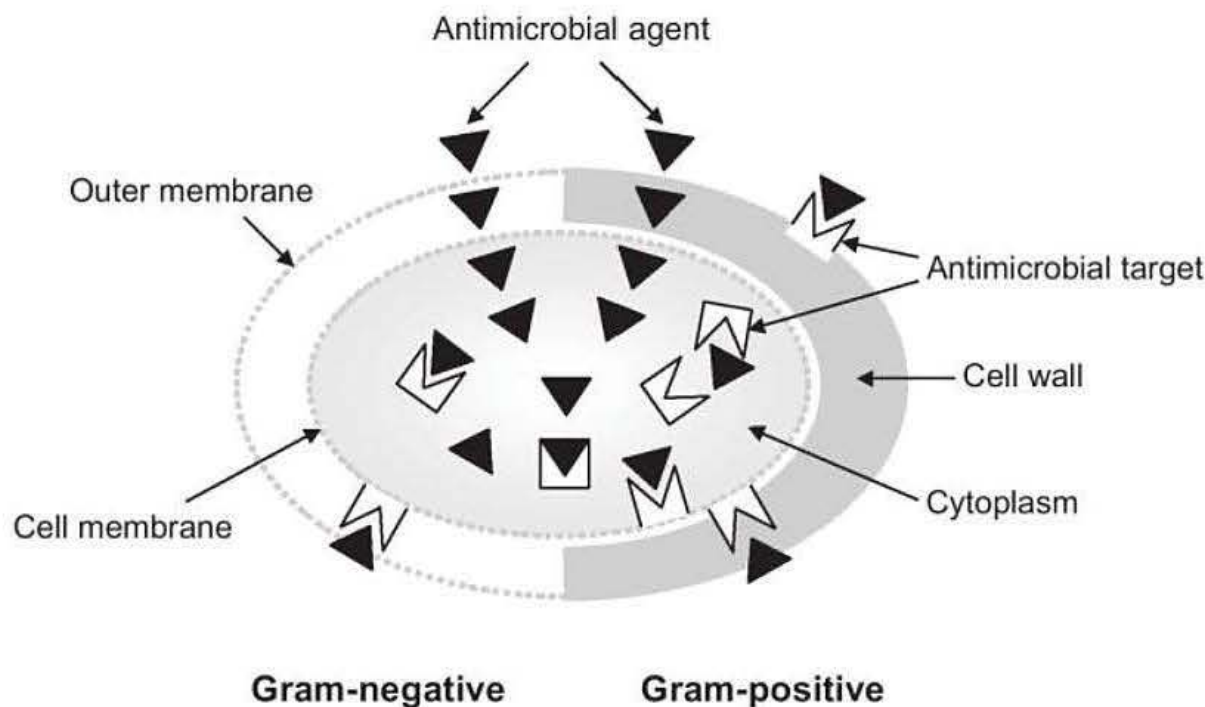


Figure 3.1. The four major mechanisms of antimicrobial resistance.

1A. The basic targets of antimicrobial agents in Gram-negatives (located mainly in the cell membrane and intracellular compartment) and Gram-positive bacteria (located mainly in the cell wall and intracellular compartment).

Intrinsic resistance is natural to all the members of a specific bacterial taxonomic group (genus, species or subspecies), and results from structural or biochemical characteristics inherent to the wild-type microorganism. For instance, Gram-negative bacteria are naturally resistant to the activity of macrolides because these agents are too large to traverse the cell wall and gain access to their cytoplasmic target. Other examples include the general reduced activity of aminoglycosides against anaerobes, which is due to poor drug penetration into the cells under anaerobic conditions; or polymyxin resistance among Gram-positive bacteria, which lack the biological target (phosphatidylethanolamine in the cytoplasmic membrane). Examples of important intrinsic resistances are presented in Table 3.2. These intrinsic resistances should be known by clinicians and other users of antimicrobial agents in order to avoid inappropriate and ineffective therapy.

Antimicrobial resistance can also be acquired, often through genetic change in a normally susceptible organism. Acquisition of resistance usually leads to discrete jumps in the MIC of an organism and hence to

clear bi- or polymodal MIC distributions (Figure 3.2). However, in some instances, such as for fluoroquinolone antimicrobials, acquisition of resistance (elevated MICs) may be a progressive phenomenon (successive accumulation of multiple genetic modifications) in which step-wise mutations occur in particular topoisomerase genes (Hopkins et al., 2005) (Table 3.3).

Acquired resistance can be to a single agent, to some but not all agents within an antimicrobial class, to a whole class of antimicrobial agents, or even to agents of several different classes. A single broad-spectrum mechanism (such as a multidrug efflux system) or simultaneous acquisition of several unrelated genetic determinants may result in broad resistance. For instance, resistance to one fluoroquinolone often results in partial or full resistance to other members of the class (Hooper, 1999).

It should be clear from the above that the acquisition of genetic determinants of resistance is associated with a range of MIC changes and does not always lead to clinically relevant resistance. Therefore, the use of MICs (or at least the distinction between microbiolog-

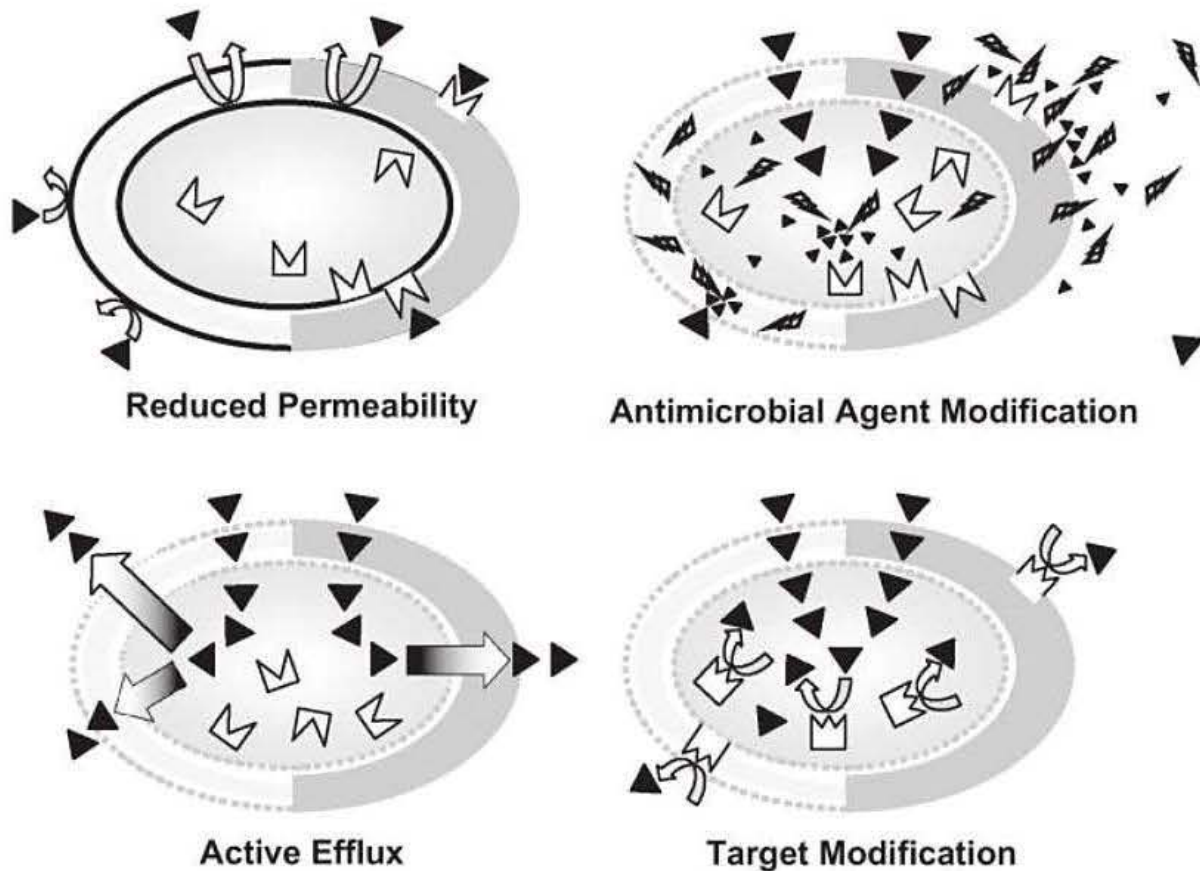


Figure 3.1B. Reduced permeability can result from either lack of permeability of the outer membrane (for instance, down-regulation of porins in Gram-negatives) or of the cell membrane (for instance, lack of aminoglycoside active transport under anaerobic conditions). Active efflux can pump antimicrobial agents back into the periplasmic space (as with the TetA tetracyclines efflux pump in Enterobacteriaceae) or directly in the outer milieu (as for the RND [Resistance/Nodulation/cell Division] multidrug efflux transporters). Antimicrobial agent modification by bacterial enzymes can take place either after the agent has penetrated into the cell (for instance, acetylation of chloramphenicol by CAT [Chloramphenicol Acetyl Transferase] enzymes), in the periplasmic space (for instance, splitting of the β -lactam ring by β -lactamases in Enterobacteriaceae), or even outside of the bacterial cell (for instance, β -lactamase produced by *Staphylococcus aureus*), before the agent has reached its target on the surface of the bacterium. Target modification has been described for both surface-exposed (for instance, peptidoglycan modification in vancomycin-resistant enterococci) and intracellular targets (for instance, macrolide resistance due to ribosomal methylation in Gram-positive bacteria).

ical resistance mechanisms and clinical responses), rather than categorical classification into resistant and susceptible, is encouraged. This would avoid many misunderstandings between clinicians and microbiologists in setting appropriate susceptibility and resistance breakpoints.

Acquisition of Antimicrobial Resistance

Bacterial antibiotic resistance can result from the mutation of genes involved in normal physiological proc-

esses and cellular structures, from the acquisition of foreign resistance genes, or from a combination of these two mechanisms. Mutations occur continuously but at relatively low frequency in bacteria, leading to the occasional random emergence of resistant mutants. However, under conditions of stress (including those encountered by pathogens when facing host defenses and antimicrobials), bacterial populations may increase their mutation frequencies (Blazquez, 2003; Chopra et al., 2003). This so-called mutator state may be involved in the rapid development of resistance *in vivo* during treatment with antimicrobials such as flu-

Table 3.1. Examples of resistance mechanisms.

Antimicrobial agent	Resistance mechanism	Examples of genetic determinant
Tetracycline	2. Inducible efflux of tetracycline in <i>E. coli</i> and other Enterobacteriaceae	<i>tetA</i> , <i>tetB</i> , <i>tetC</i>
Chloramphenicol	4. Ribosomal protection in Gram-positive bacteria	<i>tetO</i> , <i>tetM</i>
	2. Efflux in Enterobacteriaceae	<i>cmlA</i> , <i>flaR</i>
β -lactams	3. Acetylation in Enterobacteriaceae	<i>catA</i>
Oxacillin, methicillin	3. β -lactamases in Enterobacteriaceae, <i>Staphylococcus aureus</i>	<i>bla_{TEM}</i> , <i>bla_{SHV}</i> , <i>bla_{CMY-2}</i> , <i>bla_z</i>
Imipenem	4. Alternate penicillin-binding proteins in <i>Staphylococcus aureus</i>	<i>mecA</i>
Aminoglycosides	1. Decreased porin formation in <i>Enterobacter aerogenes</i> and <i>Klebsiella</i> spp.	Mutations
	3. Phosphorylation, adenylation, and acetylation of aminoglycosides in Gram-negative and -positive bacteria	Numerous genes with a broad variety of specificities
Streptomycin	4. Modification of ribosomal proteins or of 16S rRNA in <i>Mycobacterium</i> spp.	Mutations
Macrolides, Lincosamides, streptogramins	4. Methylation of ribosomal RNA in Gram-positive organisms	<i>ermA</i> , <i>ermB</i> , <i>ermC</i>
Macrolides, streptogramins	2. <i>Staphylococcus</i> spp.	<i>vga(A)</i> , <i>msr(A)</i>
Fluoroquinolones	4. DNA topoisomerases with low affinity to quinolones	Mutations in <i>gyrA</i> , <i>gyrB</i> , <i>parC</i> , <i>parE</i>
Sulfonamides	4. Bypass of blocked pathway through additional resistant dihydropteroate synthase in Gram-negative bacteria	<i>sul1</i> , <i>sul2</i> , <i>sul3</i>
Trimethoprim	4. Bypass of blocked pathway through additional resistant dihydrofolate reductase	Diverse <i>dhfr</i> genes

Note: This is by no means a comprehensive list of all the resistance mechanisms for each category of antimicrobials listed. Numbers 1, 2, 3 and 4 refer to mechanisms listed in text.

Commensal bovine and porcine *E. coli*

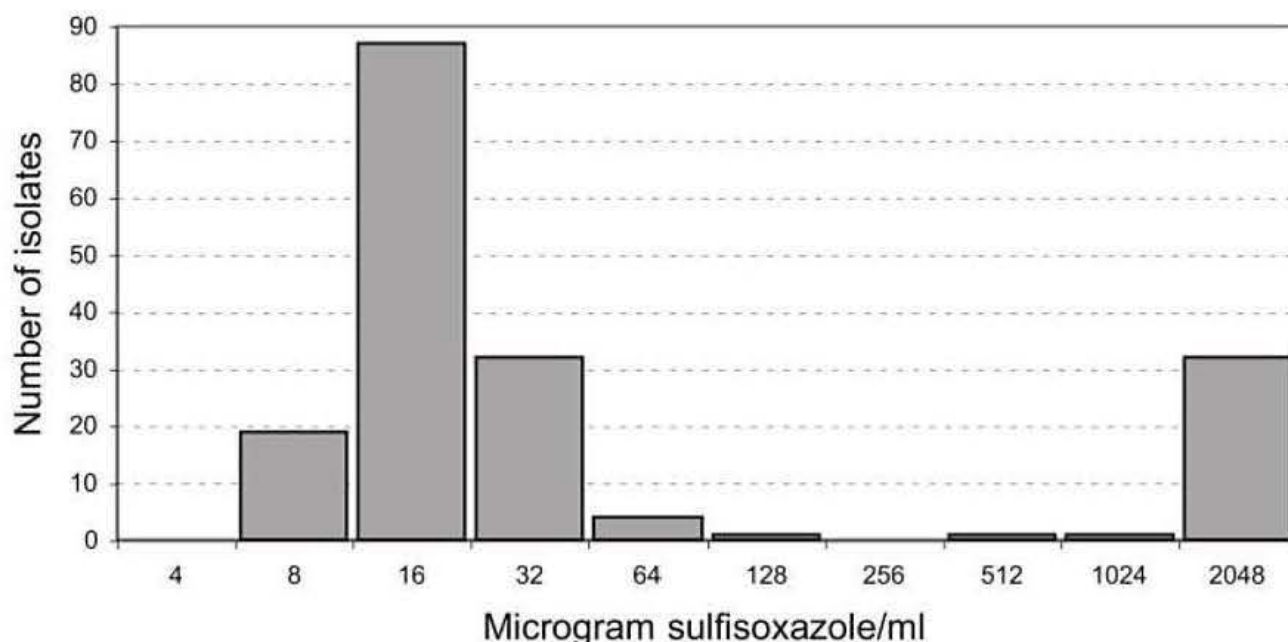


Figure 3.2. MIC Distribution for sulfisoxazole among commensal *Escherichia coli* isolates from swine and cattle. The histogram shows a clear gap between fully susceptible isolates (left) and isolates which possess a *sul*-resistance gene (right).

Table 3.2. Examples of intrinsic resistance phenotypes.

Organism	Intrinsic resistance
Most Gram-negative bacteria (<i>Enterobacteriaceae</i> , <i>Pseudomonas</i> spp.)	Penicillin G, oxacillin, macrolides, lincosamides, streptogramins, glycopeptides, bacitracin
<i>Klebsiella</i> spp.	Ampicillin
<i>Proteus vulgaris</i>	Ampicillin, cephalosporins I, polymyxins
<i>Proteus mirabilis</i>	Tetracycline, polymyxins
<i>Serratia marcescens</i>	Ampicillin, Amoxicillin-clavulanate, cephalosporins I, polymyxins
<i>Enterobacter</i> spp.	Ampicillin, Amoxicillin-clavulanate, cephalosporins I, cefoxitin
<i>Pseudomonas aeruginosa</i>	Ampicillin, cephalosporins I and II, ceftriaxone, kanamycin, tetracycline, chloramphenicol, trimethoprim, quinolones
<i>Haemophilus</i> spp.	Streptomycin, kanamycin, macrolides
<i>Campylobacter jejuni</i> and <i>Campylobacter coli</i>	Cephalosporins I, trimethoprim
Most Gram-positive bacteria	Polymyxins, quinolones
<i>Streptococcus</i> spp.	Aminoglycosides (low level)
<i>Enterococcus</i> spp.	Oxacillin, cephalosporins, aminoglycosides (low level), sulfonamides (<i>in vivo</i>), trimethoprim (<i>in vivo</i>)
<i>Listeria monocytogenes</i>	Oxacillin, cephalosporins, lincosamides
<i>Bacillus anthracis</i>	Cephalosporins, sulfonamides, trimethoprim
Anaerobes (including <i>Clostridium</i> spp.)	Aminoglycosides

Adapted from the Communiqué 2005 of the Comité de l'Antibiogramme de la Société Française de Microbiologie.

Table 3.3. Characterization of quinolone-resistant avian pathogenic *E. coli* ($n=56$).^a

No. of isolates	Mutation in ^b			MIC range ($\mu\text{g/ml}$) ^c			
	GyrA	GyrB	ParC	Nal	Orb	Enr	Cip
40	Ser83-Leu	None	None	64->256	0.5-8	0.25-2	0.12-1
7	Asp87-Tyr	None	None	128	0.5-1	0.25-0.5	0.12-0.25
1	Asp87-Tyr	None	Ser80-Ile	>256	>16	16	8
1	Ser83-Leu; Asp87-Gly	None	None	128	1	0.5	0.25
1	Ser83-Leu; Asp87-Ala	None	None	>256	2	1	0.5
1	Ser83-Leu; Asp87-Gly	None	Ser80-Arg	>256	8	4	2
2	Ser83-Leu	Asp426-Thr	None	256	2	0.5	0.25-0.5
1	Ser83-Leu	Glu466-Asp	None	>256	8	2	1
1	Ser83-Leu	Glu466-Asp	Ser80-Ile	>256	>16	8	4
1	Ser83-Leu	Glu466-Asp	Ser80-Ile	>256	>16	8	4

^aAdapted from S. Zhao, et al. 2005. Antimicrobial susceptibility and molecular characterization of avian pathogenic *Escherichia coli* isolates. *Vet Microbiol.* 107:215.

^bSubstituted amino acids, and the position number, e.g., Ser83-Leu indicates substitution of a leucine for a serine at position 83. Amino acids: Ser, serine; Asp, aspartic acid; Leu, leucine; Tyr, tyrosine; Glu, glutamic acid; Gly, glycine; I, isoleucine; Arg, arginine; Ala, alanine; Thr, threonine; None, wild-type. No mutations were identified in *parE* sequences.

^cNal, nalidixic acid; Orb, orbifloxacin; Enr, enrofloxacin; Cip, ciprofloxacin.

oroquinolones (Komp-Lindgren et al., 2003). However, for the majority of antimicrobials, resistance in clinical isolates is the result of acquisition of extra-chromosomal resistance genes.

Bacteria acquire foreign DNA in three different ways (Figure 3.3). Both the uptake of naked DNA

present in the environment by naturally competent bacteria (transformation) and the transfer of DNA from one bacterium to another by bacteriophages (transduction) have been shown to play a role in the long-term evolution of bacteria. The transfer of plasmids through a mating-like process called conjugation

represents the third mechanism of horizontal gene transfer between bacteria. Plasmids are extra-chromosomal, self-replicating genetic elements that are not essential to survival. They typically carry genes that impart some selective advantage to the host bacterium, such as antimicrobial resistance. Despite the apparent efficiency of these transfer mechanisms, bacteria avoid subversion by foreign DNA. To become established in a bacterial population, resistance genes have to overcome many more hurdles than just moving into the next cell (Thomas and Nielsen, 2005).

The paucity of documented cases of transformations and transductions associated with transfer of resistance genes, together with the plethora of examples of transferable resistance plasmids in bacterial hosts, suggest that conjugation is the major player in the global spread of antimicrobial resistance in bacterial populations. On a more local and short term scale, resistance plasmids transfer very effectively *in vivo*, leading in some cases to the emergence and establishment of newly resistant pathogen populations in individual animals within days (Poppe et al., 2005).

In addition to moving between bacteria, resistance genes can also move within the genome of a single bacterial cell and hop from the chromosome to a plasmid or between plasmids or back to the chromosome, allowing for a variety of resistance gene combinations and clusters to occur over time.

Resistance genes can also be mobilized by transposons and integrons. Transposons are genetic elements that can move from one location on the chromosome to another. The transposase genes required for movement are located within the transposon itself. The simplest form of a transposon is an insertion sequence (IS) containing only those genes required for transposition. An advancement on the IS model is seen in composite transposons: Composite transposons consist of a central region containing genes (passenger sequences) other than those required for transposition (e.g., antibiotic resistance) flanked on both sides by IS that are identical or very similar in sequence, usually in inverted orientations. A large number of resistance genes in many different bacterial species are known to occur as part of composite transposons (Salysers and Amiable-Cuevas, 1997). Some bacteria (mainly anaerobes and Gram-positive bacteria) can also carry so-called conjugative transposons, which are usually integrated in the bacterial chromosome but can be excised, subsequently behave like a

transferable plasmid, and finally reintegrate into the chromosome of their next host (Salysers et al., 1995).

The magnitude of resistance development is also explained by the recent discovery of a novel genetic system for the movement of antibiotic resistance genes called integrons (Hall et al., 1999). Integrons are DNA elements with a specific structure consisting of two conserved segments flanking a central region in which antimicrobial resistance 'gene cassettes' can be inserted. Multiple gene cassettes can be arranged in tandem, and more than 60 distinct cassettes have been identified to date conferring resistance to beta-lactams, aminoglycosides, trimethoprim, chloramphenicol, streptothricin, and quaternary ammonium compounds. Integrons are usually part of composite transposons, further increasing the mobility of resistance determinants.

The Origin of Resistance Genes and Their Movement across Bacterial Populations

Resistance genes and transfer mechanisms most likely existed long before the introduction of therapeutic antimicrobials into human and veterinary medicine. For example, antimicrobial-resistant bacteria estimated to be over 2000 years old have been isolated from deep within glaciers in Canada's high Arctic regions (Dancer et al., 1997). Resistant microorganisms have also been found among historic culture collections compiled before the advent of modern day antibiotics (Smith, 1967). It is widely believed that antibiotic resistance mechanisms arose within antibiotic-producing microorganisms as a way of protecting themselves from the action of their own antibiotics. This has been substantiated by the finding of aminoglycoside-modifying enzymes in aminoglycoside-producing organisms that display marked homology to modifying enzymes found in aminoglycoside-resistant bacteria (Davies, 1997). Webb and Davies (1993) showed that a number of antibiotic drugs are contaminated with chromosomal DNA of the antibiotic-producing organism, including identifiable antimicrobial resistance gene sequences. However, as in the case of synthetic antimicrobials such as trimethoprim and sulfonamides, preexisting genes with roles unrelated to resistance might have evolved through adaptive mutations and recombinations to function as resistance genes.

Antimicrobial resistance is a problem in veterinary

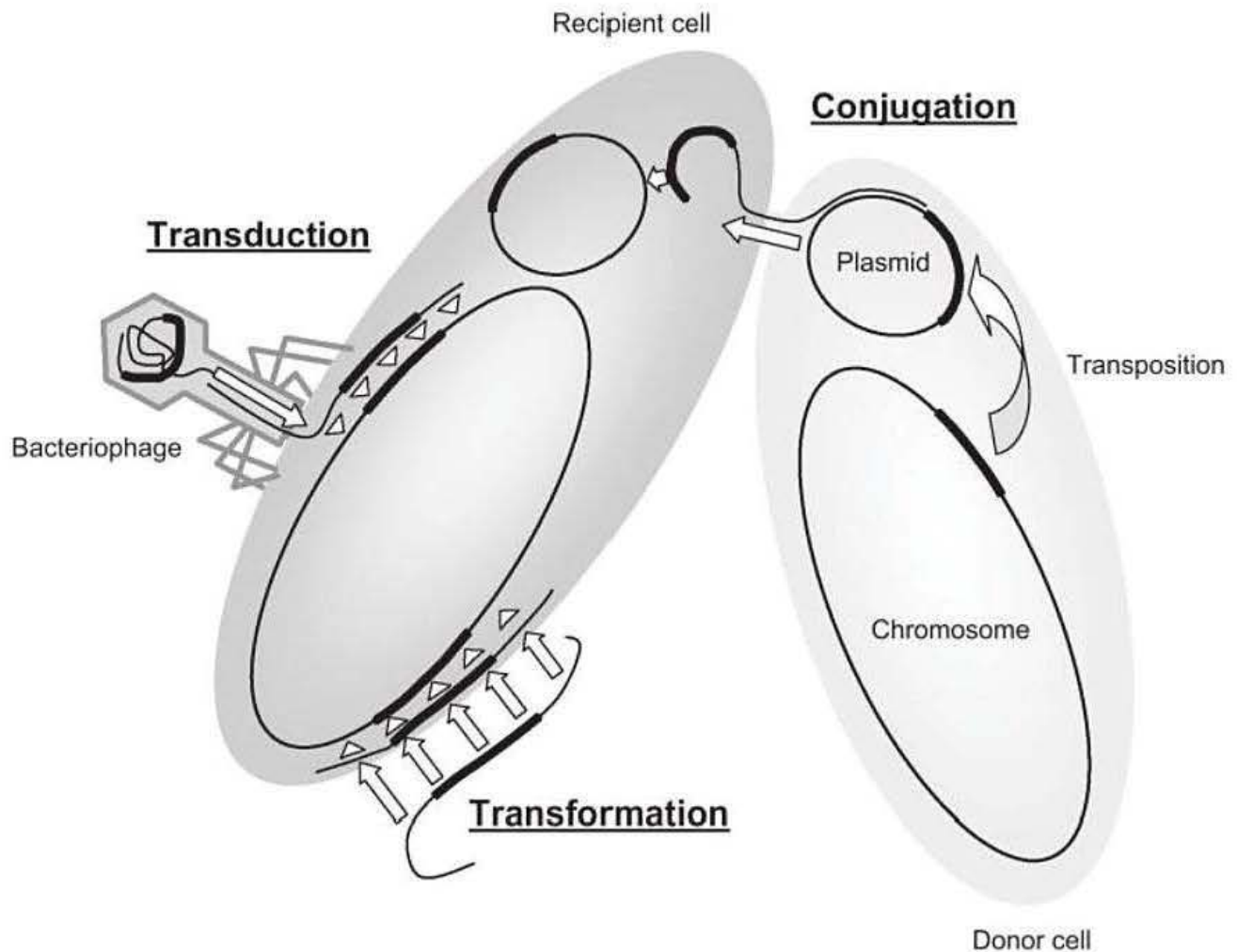


Figure 3.3. The three mechanisms of horizontal transfer of genetic material between bacteria. White arrows indicate the movement of genetic material and recombination events. Bold black lines represent antimicrobial resistance genes or gene clusters. In **transduction**, a bacteriophage injects its DNA into a bacterial cell and in the occurrence of a lysogenic phase, this DNA becomes integrated into the chromosome of the recipient cell. In **transformation**, naked DNA is taken up by a competent cell and may possibly recombine with homologous sequences in the recipient's genome. In **conjugation**, a plasmid is transferred from a donor bacterium (transfer is coupled with replication and a copy of the plasmid remains in the donor) to a recipient cell in which it can replicate. During its stay in various host bacteria, the plasmid may acquire a transposon carrying antimicrobial resistance genes.

medicine because of its implications for treatment efficacy, its cost for animal production, and its negative consequences for animal welfare. Since resistance genes are frequently located on mobile genetic elements, they can move between pathogens, as well as between non-pathogenic commensal bacteria and pathogens (Figure 3.4). Thus, the issue of resistance must be considered beyond the veterinary profession and specific pathogens. Environmental bacteria and non-pathogenic commensals may play an important

role as reservoirs or vehicles of resistance genes between pathogens. Resistance genes can spread quickly among bacteria, sometimes to unrelated genera. Even if an ingested bacterium resides in the intestine for only a short period of time, it has the ability to transfer its resistance genes to the resident microflora, which in turn may serve as reservoirs of resistance genes for pathogenic bacteria. Such exchange raises concern for the possible spread of antimicrobial resistance determinants from commensal organisms in an-

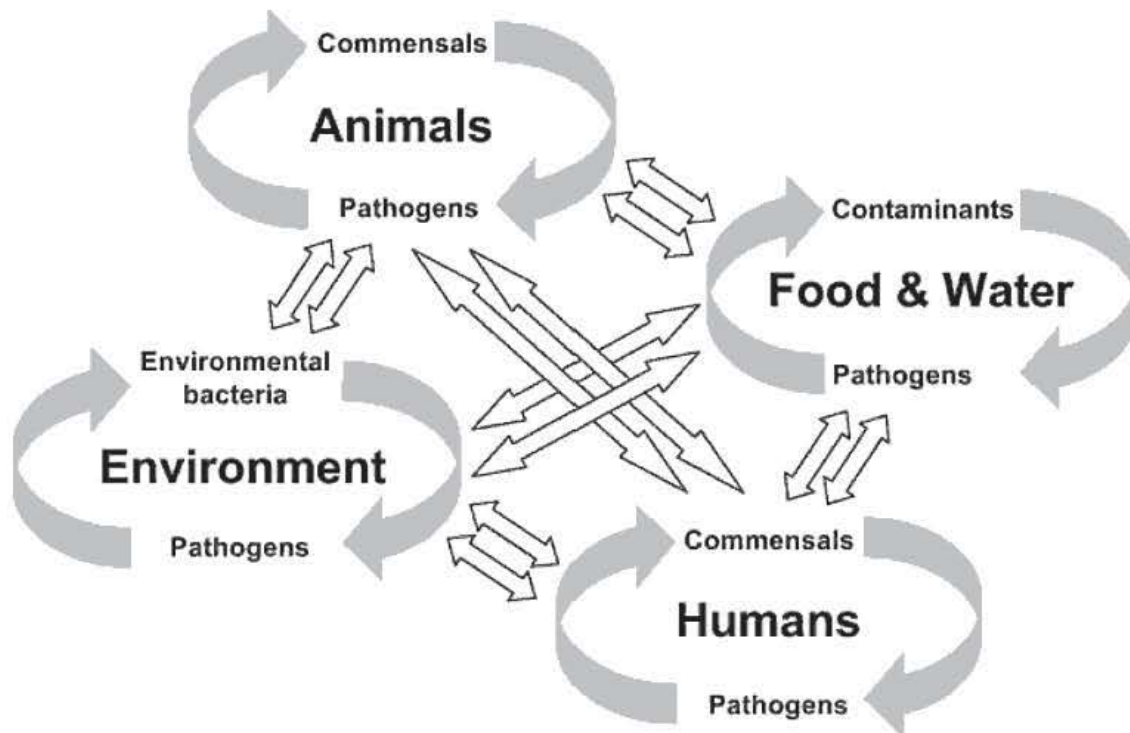


Figure 3.4. A simplified view of antimicrobial resistance epidemiology. As known from the epidemiology of zoonoses, bacteria can be transferred (white arrows) between animals and humans, through food and water, through direct contact, and through the environment. In developing antimicrobial resistance, bacteria exchange genetic material (including resistance genes) within and among each of these compartments.

imals and man to human pathogens (Witte, 1998; Van den Bogaard and Stobberingh, 2000).

There is no doubt that on a long-term evolutionary scale, the epidemiology of antimicrobial resistance should be regarded as dominated by stochastic movement of resistance genes within a gigantic bacterial genetic pool. However, on the short term and local scale, this unrestricted approach may be too simplistic and of no more relevance than considering only resistant pathogens. Thus, because of the complexity of the issue, numerous diverging strategies to control the rise of antimicrobial resistance have emerged in the scientific and medical communities.

The Effects of Antimicrobial Use on the Spread and Persistence of Resistance

The increased prevalence and dissemination of resistance is an expected outcome of natural selection, an example of the Darwinian principal of 'survival of the

fittest'. In any large population of bacteria, a few cells will possess traits that enable them to survive in the presence of a toxic substance. Susceptible organisms (those lacking the advantageous trait) will be eliminated, leaving the resistant population behind. Long-term antimicrobial use will change the microbial ecology in a given environment dramatically, with less susceptible organisms becoming the predominant population (Marshall et al., 1990; Salyers and Amabile-Cuevas, 1997; Levy, 1998). In this way, resistant commensal and opportunistic bacteria can quickly become established as components of the normal flora of various host species, displacing antimicrobial-susceptible populations.

The clustering of multiple resistance genes on plasmids, transposons, and integrons makes the problem of antimicrobial resistance even more challenging. For example, exposure to one antimicrobial may co-select for bacteria that are resistant to several unrelated agents. An additional complication is the fact that there may be non-antibiotic selection pressure for bacterial

antibiotic resistance genes. Recent data indicate that not only do resistance determinants for antibiotics of different classes aggregate, but they may also form clusters with resistance genes for non-antibiotic substances such as heavy metals and disinfectants (Salyers and Amabile-Cuevas, 1997; Hallet et al., 1999), or even with virulence genes (Boerlin et al., 2005).

Carrying and replicating resistance genes when they are not needed represents a burden for bacteria. Therefore, when a bacterial population is not under the selective pressure of antimicrobials, susceptible bacteria not carrying resistance genes may be at advantage and the population as a whole is expected to slowly revert to a mainly susceptible state. A few examples of such a reversion have been described in the past (Aarestrup et al., 2001; Boerlin et al., 2001b). However, recent work has also shown that bacteria may exhibit resistance to antimicrobials despite a lack of specific selective pressures (Bischoff et al., 2005; Johnsen et al., 2005). The exact mechanisms behind this persistence are not yet clear and are likely to be multifactorial. They may include compensation for the metabolic load imposed by resistance genes by as yet undetermined mechanisms, regulation of gene expression by the presence/absence of antimicrobials, and plasmid addiction systems. It is also clear that when resistance genes are physically linked together or to other selectively advantageous genes such as virulence determinants, co-selection will lead to the persistence of all the resistance genes as part of the cluster. Several examples of co-selection are known, such as the maintenance of glycopeptide resistance in porcine enterococci by the use of macrolides (Martinez and Baquero, 2002).

Finally, the effects of diverse drug administration protocols (administration route, timing, dosage) on the dynamics and persistence of susceptible and resistant bacteria and on the spread of resistance genes among bacterial populations at the individual and global level are still poorly understood. Every effort should be made to define protocols avoiding or minimizing selection of resistant bacteria.

Antimicrobial Resistance and Public Health

While most of the bacterial antimicrobial resistance observed in human medicine may be ascribed to use in human patients, it is resolutely argued that antimicrobial use in veterinary medicine and food animal

agriculture contributes to antimicrobial resistance in foodborne bacterial pathogens. These concerns are not new; they led in the 1960s to the release in the United Kingdom of the Swann Report (Anonymous, 1969). Despite the best efforts to date, there is still no complete agreement regarding the impact of antimicrobial use in animals on human health. The fundamental and obvious concern over the agricultural use of antibiotics arises from the potential that antimicrobials used on the farm select for resistant bacterial strains that are transferred to humans via direct contact and ingestion of contaminated food and water. Numerous cases of transmission of resistant bacteria between animals and humans at risk, such as farmers and abattoir workers, have been documented and support these concerns (Hunter et al., 1994; van den Bogaard et al., 2002). The rise and fall of resistance to glycopeptides in animal and human enterococci in some European countries paralleling the introduction and subsequent ban of avoparcin (see below) and other antimicrobial growth promoters seem to further substantiate these fears. The identification of fluoroquinolone-resistant *Campylobacter* and quinupristin/dalfopristin-resistant enterococci from animal sources or their immediate environments has intensified this debate (Piddock, 1996; Witte, 1998). Food of animal origin was even recently suggested to represent a potential reservoir of resistant uropathogenic *E. coli* for humans (Ramchandani et al., 2005). Methicillin-resistant *Staphylococcus aureus* (MRSA) seems to represent another emerging resistant zoonotic agent (see below). This suggests that, because of their intimate contact with humans, pets may represent another potential source of resistant bacteria and resistance genes of public health relevance. Prescott (2006) provides a historical perspective on the issue of agricultural use of antimicrobial drugs and its impact on human health.

Examples of Veterinary Antimicrobial Resistance of Public Health Significance

Resistance in *Salmonella*

Although a large body of scientific data is available on the prevalence of antimicrobial resistance and associated mechanisms in *Salmonella*, many aspects related to the emergence, persistence and dissemination of antimicrobial resistance in these pathogens remain unclear.

Salmonella can colonize and cause disease in a vari-

ety of food-producing and non-food-producing animals and currently includes more than 2,500 different serotypes, some of which are highly adapted to specific animal hosts (e.g., *S. dublin* in cattle, *S. gallinarum* in poultry). Although all serotypes may be regarded as potential human pathogens, the vast majority of infections are caused by a very limited number of serotypes. As with many bacterial pathogens, antimicrobial resistance in non-typhoidal *Salmonella* spp. has become an international concern (Threlfall, 2000; Poppe et al., 2001; Williams, 2001). The levels and extent of resistance vary in different places, and are influenced by antimicrobial use practices in humans and animals, as well as geographical differences in the epidemiology of *Salmonella*. In developed countries, drug resistance phenotypes in *Salmonella* have been associated with the use of antimicrobials in food-producing animals (Pidcock, 1996; Wiuff et al., 2000; Molbak, 2004; Alcaine et al., 2005). Resistance profiles generally reflect the length of time an agent has been in use. Thus, irrespective of source (food animals, food, humans), the most frequent resistances are for older agents such as tetracycline, streptomycin, sulfamethoxazole, ampicillin and chloramphenicol (Anderson, 1968; Chiappini et al., 2002; Molbak, 2004; Sun et al., 2005). However, there have been increasing reports of *Salmonella* isolates worldwide displaying reduced susceptibility or resistance to extended-spectrum cephalosporins or fluoroquinolones (Threlfall et al., 2000; Zhao et al., 2001; Gupta et al., 2003; Alcaine et al., 2005; Johnson et al., 2005). This is particularly troublesome since these two antimicrobial classes are frequently used in treating *Salmonella* infections in children and adults, respectively (Angulo et al., 2004; Alcaine et al., 2005).

Among the over 1,500 serovars identified within *S. enterica* subsp. *enterica* isolates, *S. typhimurium* continues to be one of the most frequently recovered from food animals worldwide (Zhao et al., 2005). In the United States, it is among the top four most frequently detected *Salmonella* serotypes in cattle, swine, chickens, and turkeys. Because of this broad host range, *S. typhimurium* is also one of the most common serotypes isolated from human clinical cases of salmonellosis.

An increase in the incidence of *Salmonella enterica* serotype Newport infections was initially reported by the US Centers for Disease Control in 2000. Many strains exhibited a multidrug-resistant phenotype

(commonly referred to as *S. newport* MDR-AmpC) characterized by resistance to nine antimicrobials: ampicillin, amoxicillin-clavulanic acid, cefoxitin, cephalothin, ceftiofur, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline. In addition to the characteristic resistance to nine antimicrobials, these strains also exhibited decreased susceptibility to ceftriaxone (MIC 16-32 µg/ml) (Zhao et al., 2003). These strains are of particular clinical concern, as they possess plasmid-mediated or chromosomally encoded AmpC beta-lactamases (e.g., *bla_{CMY}*) which confer decreased susceptibility to a wide range of beta-lactams, including ceftriaxone, the drug of choice for treating complicated salmonellosis in children (Gupta et al., 2003). *Salmonella newport* MDR-AmpC strains have been linked to human exposure via dairy cattle, and foodborne transmission in contaminated ground beef, pork, and other food products (Gupta et al., 2003; Zhao et al., 2003). It is important to also point out non-food animal sources of salmonellosis. Multidrug-resistant *Salmonella* strains from both equine and companion animal veterinary facilities have been associated with illness in animals and humans (Wright et al., 2005). These reports frequently describe poor hand-washing practices by employees, eating in work areas, and previous antimicrobial drug therapy in humans or animals.

Since *Salmonella* serovars Typhimurium and Newport very often exhibit multidrug-resistant phenotypes (Threlfall et al., 2005), tracking of antimicrobial susceptibility profiles, as well as molecular genetic types, is essential in characterizing outbreaks and guiding anti-infective therapy if warranted.

Methicillin-resistant *Staphylococcus aureus* (MRSA)

MRSA has emerged as a major nosocomial pathogen in human hospitals, with few alternatives for antimicrobial treatment. Until recently, this problem was limited to hospital settings, but MRSA has now started to spread in the human community at large. Though once rare, MRSA infection is increasingly reported in animals (Duquette and Nuttall, 2004; Middleton et al., 2005). Most such reports are from horses, dogs, and cats, presumably because of their close contact with people, who are the source of the infection. MRSA isolates were first recovered from horses in relation with nosocomial surgical wound infections possibly originating from humans (Seguin et al., 1999). Ongoing studies of equine MRSA in Ontario, Canada and the

northeastern United States have shown that equine MRSA from that region usually belongs to a single clone, which seems to be maintained within equine populations (Weese et al., 2005a, 2005b). This clone is also occasionally found in humans, particularly in horse personnel, but does not seem to belong to one of the human MRSA clones currently most prevalent in this region. These investigations suggest bi-directional transmission of MRSA between humans and horses, but much work remains to clarify the epidemiology of MRSA in horses and its public health potential. However, under particular circumstances, transmission from horse to human has resulted in clinical infection.

The epidemiology of MRSA in dogs and cats is not yet well understood. However, the MRSA clones found in dogs and cats and occasionally transmitted from one animal to the next are the same as those frequently found in nosocomial and community infections in humans. In addition, many reports show that the same MRSA strain from clinical infections or from healthy carriage can frequently be found in pets and humans with close contact (van Duijkeren et al., 2004a, 2004b, 2005; O'Mahony et al., 2005; Rankin, 2005). In one specific case, MRSA in humans could only be eliminated after it had been eradicated from their pet dog (Manian, 2003). Thus, the MRSA situation in pets should be monitored seriously and may possibly become of great public health importance in the near future.

Despite the importance of *S. aureus* as an agent of mastitis and the selective pressure exerted by cloxacillin and oxacillin in its treatment and prevention, MRSA has remained a rarity in cattle. This is very fortunate, because of the potential significance for public health and food safety that MRSA could have, if found in milk. This situation must be monitored regularly and the lack of a link between *S. aureus* from dairy cattle and from humans clarified further.

Antimicrobials in Animal Feeds and Association with Resistance in Bacteria of Human Health Significance

It has been known for decades that continuous oral administration of low concentrations of antimicrobials increases feed conversion and weight gain, and reduces shipping stress-associated diseases in food animals (Butaye et al., 2003; Dibner and Richards, 2005). This practice is also a potentially significant driving force in accelerating the emergence of resistant bacte-

ria that could infect humans (Wegener, 2003; Kelly et al., 2004; Dibner and Richards, 2005). The use of antimicrobial agents for growth promotion is discussed in Chapter 24.

Most classes of antimicrobials used in animals have human analogues, and are therefore capable of selecting for resistance to human antibiotics. The important exceptions are the ionophores (e.g., monensin, narasin, salinomycin, lasalocid), the quinoxalines (e.g. olaquinox), bambermycins (flavophospholipol) and avilamycin (Turnidge 2004). Among those agents with human analogues, two classes of antimicrobials that have received particular scientific attention in recent years are the streptogramins and glycopeptides. Virginiamycin and quinupristin/dalfopristin are examples of streptogramins, whereas avoparcin and vancomycin are glycopeptides.

Since 1975, virginiamycin has been an approved food-animal feed additive for growth promotion and disease prevention or control in turkeys, swine, cattle and chickens (Kelly et al., 2004). The human analogue, Synercid®, a mixture of the two streptogramin antibiotics quinupristin and dalfopristin (QD), was approved in September 1999 by the US FDA for treatment of bacteremias in humans, particularly against vancomycin-resistant *Enterococcus faecium* (VREF) and for the treatment of skin and soft tissue infections caused by *Staphylococcus aureus* and *Streptococcus pyogenes*. At the time of approval, Synercid was considered a therapy of last resort for potentially life-threatening bloodstream infections (BSIs) caused by VREF. The approval of Synercid focused increased attention on the use of virginiamycin in animal husbandry; specifically, whether farm use of this antimicrobial analogue resulted in the development of streptogramin resistance in bacteria that could impair Synercid therapy in humans (Wegener 2003; Kelly et al. 2004). Since virginiamycin induces cross-resistance to Synercid, it has been postulated that the use of virginiamycin in animal husbandry might influence the prevalence of Synercid resistance among human *E. faecium* isolates as a result of transmission through the food supply. Synercid-resistant *E. faecium* (SREF) is common in the poultry production environment, including samples from litter and transport containers. SREF is also common on poultry meat products at retail, suggesting that such meats serve as a continual source of resistant strains and/or their resistance genes (McDermott et al., 2005). This raises the possibility that foodborne

strains may transfer plasmid-borne resistance determinants to human native enterococci in vivo (Jacobsen et al., 1999), which in turn may donate these genes to other strains, causing human infections. The food safety implications have prompted the US Food and Drug Administration (<http://www.fda.gov/OHRMS/DOCKETS/98fr/04-25979.pdf>) and others (Cox and Popken, 2004; Kelly et al., 2004) to propose risk assessment models examining the potential public health consequences of virginiamycin use. However, at this time, the potential for streptogramin resistance genes to transfer from foodborne enterococcal isolates to those causing disease in humans remains largely unknown. Therefore, estimations of the potential health risks to humans resulting from virginiamycin use in animal husbandry will need updating as more information on the genetics of resistance becomes available.

Early studies in the 1990s provided evidence in favor of a causal association between the use of avoparcin and the occurrence of VREF on farms in Europe (Bager et al., 1997; Aarestrup et al., 2000). This suggested that food animals constitute a potential reservoir of infection for VREF in humans (Wegener, 2003). In response to continued pressure from the 'major harm' position, the European Union adopted the 'precautionary principle' and followed the earlier move of Scandinavian countries by suspending the use of the 'growth promoter' in-feed antibiotics: Avoparcin, virginiamycin, spiramycin, tylosin and bacitracin were banned from feed because of their ability to select for resistance to antimicrobials of human importance (Turnidge, 2004). The frequency of resistance to vancomycin and to growth promoters in enterococci of animal origin has been shown to generally decline at least to some degree after the ban of antimicrobial growth promoters (Aarestrup et al., 2001; Boerlin et al., 2001b; Sorum et al., 2004). Interestingly, the linkage of glycopeptide and macrolide resistance genes in VREF slowed the decrease of VREF frequency in swine until tylosin was also banned as a growth promoter (Aarestrup et al., 2001). Some studies have also demonstrated a parallel trend in enterococci isolated from food and humans, strongly supporting the usefulness of the ban (Klare et al., 1999; Pantosti et al., 1999). However, VREF persists in animals (Borgen et al., 2000; Heuer et al., 2002), and isolates similar to those from animals could be recovered from humans several years after the ban of avoparcin (Hammerum et al., 2004). Thus, the antimicrobial resistance associ-

ated with the use of antimicrobial growth promoters is not going to vanish as quickly as early studies had led us to hope. In addition, the global ban of antimicrobial growth promoters might have undesirable consequences on animal health and on the use of therapeutic antimicrobials, which remain to be assessed precisely (Casewell et al., 2003).

Surveillance Programs and the Role of Diagnostic Laboratories

The seriousness of the antimicrobial resistance threat has prompted many governments to initiate surveillance programs that include bacteria of animal origin. These programs provide a tool to globally assess the extent of the problem, to follow its evolution over time, and to evaluate the effectiveness of control measures. Such systems include, among others, the National Antimicrobial Resistance Monitoring System (NARMS) and the Collaboration in Animal Health, Food Safety & Epidemiology (CAHFSE) network in the United States, the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) in Canada, and the Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP) in Denmark. On the veterinary side, some include only bacteria considered indicators of the general resistance situation (i.e., *Escherichia coli* and *Enterococcus* spp.) and zoonotic bacterial agents (*Salmonella enterica* and *Campylobacter* spp.). Some programs such as DANMAP and CAHFSE also include a small number of specific animal pathogens. Surveillance programs are of particular interest when, like DANMAP, they include the collection of data on antimicrobial use and try to link this with the evolution of resistance. Because of the past problems in comparability associated with a lack of standardization in antimicrobial susceptibility testing, it is also encouraging to see that these national surveillance programs use similar (if not identical) methodologies and provide increasingly comparable data.

There is a wealth of information in the scientific literature on the prevalence of antimicrobial resistance in animal pathogens (Aarestrup, 2006). However, because of the geographically local and temporarily limited nature of these studies, with very different sampling and susceptibility testing methodologies, it is difficult to draw reliable conclusions on the global antimicrobial resistance situation in veterinary medicine. Constant efforts are made by the Clinical and

Laboratory Standards Institute (CLSI, former NCCLS) to develop agreeable veterinary standards for susceptibility testing methodologies (Chapter 2). However, in-depth investigations show that many laboratories do not strictly follow these standards. There is a great need for diagnostic laboratories to adhere to them in order to provide reliable and reproducible susceptibility data for clinicians and other users. In addition, most studies on antimicrobial resistance in veterinary pathogens are not based on a representative sample of pathogen populations, but on diagnostic laboratory submissions.

Susceptibility testing of clinical isolates is a cornerstone for prudent use of antimicrobials and adequate management of single clinical cases (Chapter 2). Unfortunately, microbiological analysis and susceptibility testing are often performed only when a problem persists after initial empirical antimicrobial therapy. Thus susceptible pathogens, which can be treated successfully, are less frequently tested in diagnostic laboratories, and the use of laboratory submissions for surveillance will inevitably overestimate the prevalence of resistance. Consequently, better designed studies are needed to assess the real antimicrobial resistance situation in veterinary pathogens at every level, from the farm all the way up to the national and international level.

Nosocomial Infection and Antimicrobial Resistance in Veterinary Hospitals

Because of the high selection pressure exerted by heavy use of antimicrobial agents in human hospitals, resistance emerged first as a significant problem in bacteria associated with nosocomial infections. As veterinary hospitals and their intensive care units increase in size and companion animal medicine continuously intensifies, antimicrobial resistance problems similar to those in human hospitals are starting to appear. However, few publications are currently available on the topic of nosocomial infections with multiresistant pathogens in animals.

Besides the emerging problem with MRSA in horses and companion animals mentioned above, other multiresistant bacterial nosocomial pathogens have been reported in veterinary hospitals, including *E. coli* (Sanchez et al., 2002), *Acinetobacter baumannii*, and *Salmonella enterica*. In addition, infections with mul-

tiresistant enterococci seem to be on the rise in veterinary intensive care units.

Multiresistant *Salmonella* is one of the most regularly encountered causes of nosocomial infections in veterinary hospitals. Equine clinics seem to be particularly at risk (Dargatz and Traub-Dargatz, 2004). However, multiresistant *Salmonella* outbreaks also happen in companion animal clinics, and transmissions to humans can occur in such cases (Wright et al., 2005). This, too, may become a public health concern.

As in human hospitals, nosocomial infections in veterinary hospitals are frequently due to multiresistant bacteria, which persist better in this harsh environment than susceptible organisms. This was, for example, the case in a series of *A. baumannii* infections in a companion animal hospital, in which persistent strains were multiresistant, whereas sporadic ones presented only few resistances. After eradication of the multiresistant strain through enforcement of hygienic measures, another persistent multiresistant strain readily replaced the first and even found its way into the neighboring equine clinic (Boerlin et al., 2001a).

Despite the paucity of scientific veterinary literature on the subject, there is a growing awareness that antimicrobial resistance and proper antimicrobial use in animal hospitals should be a concern for veterinarians. A number of university veterinary hospitals have developed and currently apply guidelines for prudent use of antimicrobials within their institutions. A more general use of these guidelines outside academic circles should be promoted.

Accumulation and Persistence of Antimicrobial Resistance in Pathogens

Resistance gene linkage and co-selection are among the reasons for the accumulation and persistence of resistance in bacterial populations (Bischoff et al., 2005; Johnsen et al., 2005). However, these do not in themselves explain why pathogens are more frequently resistant to antimicrobials than are normal flora. The most frequently cited explanation for this difference is the higher selection pressure exerted on pathogens by repeated treatments. However, linkage of resistance and virulence genes on plasmids is likely to be an additional factor explaining the higher prevalence of resistance among pathogens. Such linkages have already

been sporadically described (Martinez and Baquero, 2002), and cumulative evidence from molecular epidemiology studies shows that resistance/virulence linkage may be a relatively widespread phenomenon, at least in such organisms as *E. coli*. For instance, tetracycline resistance genes are frequently linked to enterotoxin genes in enterotoxigenic *E. coli* (ETEC), which may explain why tetracycline resistance is more frequent in ETEC than in commensal *E. coli* populations (Boerlin et al., 2005). Similarly, the linkage of chloramphenicol resistance genes to enterotoxin genes may partially explain why, despite the ban of chloramphenicol approximately two decades ago, chloramphenicol resistance is still so widespread in porcine ETEC, but less frequent in commensal *E. coli*.

One must wonder if the reverse may not also be true: Are virulence genes accumulating in bacterial populations because of their linkage with resistance genes and the selection exerted by antimicrobial use? Further studies on this topic are warranted, and if confirmatory, may have tremendous implications for the future use of antimicrobial agents.

The Control of Antimicrobial Resistance

It is questionable whether new classes of antimicrobial agents will be available for veterinary use in the coming years. The use of novel antimicrobials is likely to be restricted to human medicine, and economic considerations limit any attempt to develop new antimicrobials for veterinary use only. Thus, the antimicrobials available to the veterinary profession in the near future will probably remain much the same as today.

Therefore, continued efforts should be made to preserve their efficacy. Many professional associations, governmental agencies worldwide, and international committees are developing guidelines for the responsible and prudent use of antimicrobial agents in veterinary medicine and agriculture (Chapter 27). For example, the Office International des Epizooties (OIE) (Anthony et al., 2001), American Veterinary Medical Association (AVMA), and numerous producer and veterinary practitioner groups have developed programs to help veterinarians and producers make sound decisions about prudent and judicious antimicrobial drug use in animal production. Additionally, economic incentives such as the development of new markets for organic and 'antibiotic-free' animal prod-

ucts may help further reduce the use of antimicrobial agents in animals.

Maintenance and improvement of good management practices in companion animal medicine and food animal husbandry are cornerstones in the reduction of antimicrobial use and the control of antimicrobial resistance. Additionally, the role of alternatives to antimicrobials such as vaccines and pre- and probiotics remains to be thoroughly assessed and defined.

In conclusion, the optimism of the early period of antimicrobial discovery has been tempered by the emergence of bacterial strains resistant to these therapeutics. Today, clinically important bacteria exhibit not only single-drug resistance but also multiple antibiotic resistances, the legacy of past decades of antimicrobial use and misuse. This modern predicament of widespread antimicrobial resistance has led the World Health Organization to warn that the benefits of these agents may be lost without comprehensive and concerted action to combat the present problem and reverse anticipated developments. It must be remembered that resistance is an inevitable biological phenomenon: the challenge is to circumvent this persistent and serious obstacle to effective medical and veterinary chemotherapy.

Bibliography

- Aarestrup FM, et al. 2000. Associations between the use of antimicrobial agents for growth promotion and the occurrence of resistance among *Enterococcus faecium* from broilers and pigs in Denmark, Finland, and Norway. *Microb Drug Resist* 6:63.
- Aarestrup FM, et al. 2001. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. *Antimicrob Agents Chemother* 45:2054.
- Aarestrup, FM (ed). 2006. *Antimicrobial Resistance in Bacteria of Animal Origin*. Washington, DC: ASM Press.
- Alcaine SD, et al. 2005. Ceftiofur-resistant *Salmonella* strains isolated from dairy farms represent multiple widely distributed subtypes that evolved by independent horizontal gene transfer. *Antimicrob Agents Chemother*, 49:4061.
- Anderson ES. 1968. Drug resistance in *Salmonella typhimurium* and its implications. *Brit Med J* 3:333.
- Angulo FJ, et al. 2004. Evidence of an association between use of antimicrobial agents in food animals and antimicrobial resistance among bacteria isolated from humans and the human health consequences of such resistance. *J Vet Med B Infect Dis Vet Pub Health* 51:374.
- Anonymous. 1969. Joint Committee on the Use of Antimicrobial Drugs in Animal Husbandry and Veterinary Medicine. Her Majesty's Stationary Office, London.

- Anthony F, et al. 2001. Responsible use of antimicrobial agents in veterinary medicine. *Rev Sci Tech Office Int Epizoot* 20:829.
- Bager F. 1999. Glycopeptide resistance in *Enterococcus faecium* from broilers and pigs following discontinued use of avoparcin. *Microb Drug Res* 5:53.
- Bager F, et al. 1997. Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant *Enterococcus faecium* on Danish poultry and pig farms. *Prev Vet Med* 31:95.
- Bischoff KM, et al. 2005. The chloramphenicol resistance gene *cmlA* is disseminated on transferable plasmids that confer multiple-drug resistance in swine *Escherichia coli*. *FEMS Microbiol Lett* 243:285.
- Blazquez J. 2003. Hypermutation as a factor contributing to the acquisition of antimicrobial resistance. *Clin Infect Dis* 37:1201.
- Boerlin P, et al. 2001a. Transmission of opportunistic pathogens in a veterinary teaching hospital. *Vet Microbiol* 82:347.
- Boerlin P, et al. 2001b. Antimicrobial growth promoter ban and resistance to macrolides and vancomycin in enterococci from pigs. *J Clin Microbiol* 39:4193.
- Boerlin P, et al. 2005. Antimicrobial resistance and virulence genes of *Escherichia coli* isolates from swine in Ontario. *Appl Environ Microbiol* 71:6753.
- Borgen K, et al. 2000. Continuing high prevalence of VanA-type vancomycin-resistant enterococci on Norwegian poultry farms three years after avoparcin was banned. *J Appl Microbiol* 89:478.
- Butaye P, et al. 2003. Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on gram-positive bacteria. *Clin Microbiol Rev* 16:175.
- Casewell M, et al. 2003. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *J Antimicrob Chemother* 52:159.
- Chiappini E, et al. 2002. Results of a 5-year prospective surveillance study of antibiotic resistance among *Salmonella enterica* isolates and ceftriaxone therapy among children hospitalized for acute diarrhea. *Clin Ther* 24:1585.
- Chopra I, et al. 2003. The role of mutators in the emergence of antibiotic-resistant bacteria. *Drug Resist Updates* 6:137.
- Cox LA, Popken DA. 2004. Quantifying human health risks from virginiamycin used in chickens. *Risk Anal* 24:271.
- Dancer SJ, et al. 1997. Isolation and characterization of coliforms from glacial ice and water in Canada's high Arctic. *J Appl Bacteriol* 82:597.
- Dargatz DA, Traub-Dargatz JL. 2004. Multidrug-resistant *Salmonella* and nosocomial infections. *The Veterinary Clinics of North America. Equine Pract* 20:587.
- Davies JE. 1997. Origins, acquisition and dissemination of antibiotic resistance determinants. In: Chadwick DJ, Goode J, eds. *Antibiotic Resistance: Origins, Evolution, Selection and Spread*, Chichester, UK: John Wiley and Sons.
- Dibner JJ, Richards JD. 2005. Antibiotic growth promoters in agriculture: history and mode of action. *Poult Sci* 84:634.
- Duquette RA, Nuttall TJ. 2004. Methicillin-resistant *Staphylococcus aureus* in dogs and cats: an emerging problem? *J Small Anim Pract* 45:591.
- Gupta A, et al. 2003. Emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. *J Infect Dis* 188:1707.
- Hall RM, et al. 1999. Mobile gene cassettes and integrons in evolution. *Ann NY Acad Sci* 870:68.
- Hammerum AM, et al. 2004. A vancomycin-resistant *Enterococcus faecium* isolate from a Danish healthy volunteer, detected 7 years after the ban of avoparcin, is possibly related to pig isolates. *J Antimicrob Chemother* 53: 547.
- Helms M, et al. 2002. Excess mortality associated with antimicrobial drug-resistant *Salmonella* Typhimurium. *Emerg Infect Dis* 8:490.
- Heuer OE, et al. 2002. Vancomycin-resistant enterococci (VRE) in broiler flocks 5 years after the avoparcin ban. *Microb Drug Res* 8:133.
- Hooper DC. 1999. Mechanisms of fluoroquinolone resistance. *Drug Resist Update* 2:38.
- Hopkins KL, et al. 2005. Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: recent developments. *Int J Antimicrob Agents* 25:358.
- Hunter JE, et al. 1994. Apramycin-resistant *Escherichia coli* isolated from pigs and a stockman. *Epidem Infect* 112: 473.
- Jacobsen BL, et al. 1999. Horizontal transfer of the *satA* gene encoding streptogramin A resistance between isogenic *Enterococcus faecium* strains in the gastrointestinal tract of gnotobiotic rats. *Microb Ecol Health Dis* 11:241.
- Johnsen PJ, et al. 2005. Persistence of animal and human glycopeptide-resistant enterococci on two Norwegian poultry farms formerly exposed to avoparcin is associated with a widespread plasmid-mediated *vanA* element within a polyclonal enterococcus faecium population. *Appl Environ Microbiol* 71: 159.
- Johnson JM, et al. 2005. Antimicrobial resistance of selected *Salmonella* isolates from food animals and food in Alberta. *Can Vet J* 46:141.
- Johnston AM. 1998. Use of antimicrobial drugs in veterinary practice. *Brit Med J* 317:665.
- Kelly L, et al. 2004. Animal growth promoters: to ban or not to ban? A risk assessment approach. *Int J Antimicrob Agents* 24:7.
- Klare I et al. 1999. Decreased incidence of VanA-type vancomycin-resistant enterococci isolated from poultry meat and from fecal samples of humans in the community after discontinuation of avoparcin usage in animal husbandry. *Microb Drug Res* 5:45.
- Komp Lindgren P, et al. 2003. Mutation rate and evolution of fluoroquinolone resistance in *Escherichia coli* isolates from patients with urinary tract infections. *Antimicrob Agents Chemother* 47:3222.
- Kruse H, Sorum H. 1994. Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural environments. *Appl Environ Microbiol* 60:4015.
- Levy SB. 1998. Multidrug resistance, a sign of the times. *New Engl J Med* 338:1376.

- Manian FA. 2003. Asymptomatic nasal carriage of mupirocin-resistant, methicillin-resistant *Staphylococcus aureus* (MRSA) in a pet dog associated with MRSA infection in household contacts. *Clin Infect Dis* 36:26.
- Marshall B, et al. 1990. Inter- and intraspecies spread of *Escherichia coli* in a farm environment in the absence of antibiotic usage. *Proc Nat Acad Sci USA* 87:6609.
- Martinez JL, Baquero F. 2002. Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance. *Clin Microbiol Rev* 15:647.
- McDermott PF, et al. 2005. Changes in antimicrobial susceptibility of native *Enterococcus faecium* in chickens fed virginiamycin. *Appl Environ Microbiol* 71:4986.
- Middleton JR, et al. 2005. Surveillance of *Staphylococcus aureus* in veterinary teaching hospitals. *J Clin Microbiol* 43:2916.
- Molbak K. 2004. Spread of resistant bacteria and resistance genes from animals to humans—the public health consequences. *J Vet Med B Infect Dis Vet Pub Health* 51:364.
- National Research Council, Institute of Medicine. 1998. The use of drugs in food animals: Benefits and risks. Washington DC: National Academy Press.
- O'Mahony R, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland. *Vet Microbiol* 109:285.
- Pantosti A, et al. 1999. Decrease of vancomycin-resistant enterococci in poultry meat after avoparcin ban. *Lancet* 354:741.
- Piddock LJ. 1996. Does the use of antimicrobial agents in veterinary medicine and animal husbandry select antibiotic-resistant bacteria that infect man and compromise antimicrobial chemotherapy? *J Antimicrob Chemother* 38:1.
- Poppe C, et al. 2005. Acquisition of resistance to extended-spectrum cephalosporins by *Salmonella enterica* subsp. *enterica* serovar Newport and *Escherichia coli* in the turkey poult intestinal tract. *Appl Environ Microbiol* 71:1184.
- Poppe C, et al. 2001. Trends in antimicrobial resistance of *Salmonella* isolated from animals, foods of animal origin, and the environment of animal production in Canada, 1994-1997. *Microb Drug Res* 7:197.
- Prescott JF. 2006. History of antimicrobial usage in agriculture. In Aarestrup FM (ed). *Antimicrobial Resistance in Bacteria of Animal Origin*. pp. 19-28. Washington, DC: ASM Press.
- Ramchandani M, et al. 2005. Possible animal origin of human-associated, multidrug-resistant, uropathogenic *Escherichia coli*. *Clin Infect Dis* 40:251.
- Rankin S, et al. 2005. Panton valentine leukocidin (PVL) toxin positive MRSA strains isolated from companion animals. *Vet Microbiol* 108:145.
- Salysers AA, et al. 1995. Conjugative transposons: an unusual and diverse set of integrated gene transfer elements. *Microbiol Rev* 59:579.
- Salysers AA, Amiable-Cuevas CF. 1997. Why are antibiotic resistance genes so resistant to elimination? *Antimicrob Agents Chemother* 41: 2321.
- Sanchez S, et al. 2002. Characterization of multidrug-resistant *Escherichia coli* isolates associated with nosocomial infections in dogs. *J Clin Microbiol* 40:3586.
- Seguin JC, et al. 1999. Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: potential human-to-animal transmission. *J Clin Microbiol* 37:1459.
- Smith DH. 1967. R factor infection of *Escherichia coli* lyophilized in 1946. *J Bacteriol* 94:2071.
- Sorum M, et al. 2004. Prevalence of vancomycin resistant enterococci on poultry farms established after the ban of avoparcin. *Avian Dis* 48:823.
- Sun HY, et al. 2005. Occurrence of ceftriaxone resistance in ciprofloxacin-resistant *Salmonella enterica* serotype Choleraesuis isolates causing recurrent infection. *Clin Infect Dis* 40:208.
- Thomas CM, Nielsen KM. 2005. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. *Nature Rev Microbiol* 3:711.
- Threlfall EJ. 2000. Epidemic *Salmonella typhimurium* DT 104—a truly international multiresistant clone. *J Antimicrob Chemother* 46:7.
- Threlfall EJ, et al. 2000. Spread of resistance from food animals to man—the UK experience. *Acta Vet Scand* S93:63.
- Travers K, Barza M. 2002. Morbidity of infections caused by antimicrobial-resistant bacteria. *Clin Infect Dis*. 34:S3: S131-S134.
- Turnidge J. 2004. Antibiotic use in animals—predjudices, perceptions and realities. *J Antimicrob Chemother* 53:26.
- US Food and Drug Administration. 2004. Draft risk assessment of streptogramin resistance in *Enterococcus faecium* attributable to the use of streptogramins in animals. Federal Register 69:68384. <http://www.fda.gov/OHRMS/DOCKETS/98fr/04-25979.pdf>.
- Van den Bogaard AE, Stobberingh EE. 2000. Epidemiology of resistance to antibiotics: Links between animals and humans. *Int J Antimicrob Agents* 14:327.
- Van den Bogaard AE, et al. 2002. Antibiotic resistance of faecal enterococci in poultry, poultry farmers and poultry slaughterers. *J Antimicrob Chemother* 49:497.
- Van Duijkeren E, et al. 2004a. Methicillin-resistant staphylococci isolated from animals. *Vet Microbiol* 103:91.
- Van Duijkeren E, et al. 2004b. Human-to-dog transmission of methicillin-resistant *Staphylococcus aureus*. *Emerg Infect Dis* 10:2235.
- Van Duijkeren E, et al. 2005. Transmission of a Panton-Valentine leukocidin-positive, methicillin-resistant *Staphylococcus aureus* strain between humans and a dog. *J Clin Microbiol* 43:6209.
- Varma JK, et al. 2005. Antimicrobial-resistant nontyphoidal *Salmonella* is associated with excess bloodstream infections and hospitalizations. *J Infect Dis* 191:554.
- Webb V, Davies J. 1993. Antibiotic preparations contain DNA: a source of drug resistance genes? *Antimicrob Agents Chemother* 37:2379.
- Weese J, et al. 2005a. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel, 2000-2002. *Emerg Infect Dis* 11:430.

- Weese J, et al. 2005b. Community-associated methicillin-resistant *Staphylococcus aureus* in horses and humans who work with horses. *J Am Vet Med Assoc* 226:580.
- Wegener HC. 2003. Antibiotics in animal feed and their role in resistance development. *Curr Opin Microbiol* 6:439.
- Williams RJ. 2001. Globalization of antimicrobial resistance: epidemiological challenges. *Clin Infect Dis* 33:S3:S116.
- Witte W. 1998. Medical consequences of antibiotic use in agriculture. *Science* 279:996.
- Wiuff C, et al. 2000. Quinolone resistance among *Salmonella enterica* from cattle, broilers, and swine in Denmark. *Microbial Drug Res* 6:11.
- Wright JG, et al. 2005. Multidrug-resistant *Salmonella typhimurium* outbreak. *Emerg Infect Dis* 11:1235.
- Zhao S, et al. 2001. Identification and expression of cephamycinase *bla*CMY genes in *Escherichia coli* and *Salmonella* isolates from food animals and ground meat. *Antimicrob Agents Chemother* 45:3647.
- Zhao S, et al. 2003. Characterization of *Salmonella enterica* serotype Newport isolated from humans and food animals. *J Clin Microbiol* 41:5366.
- Zhao S, et al. 2005. Characterization of *Salmonella typhimurium* of animal origin obtained from the National Antimicrobial Resistance Monitoring System (NARMS). *Foodborne Path Dis* 2:169.

Principles of Antimicrobial Drug Bioavailability and Disposition

J. Desmond Baggot

In treating microbial infections, it is important that an effective concentration of antimicrobial drug be rapidly attained at the focus of infection and that it be maintained for an adequate duration. The concentration achieved depends on the systemic availability of the drug, which varies with the dosage form (drug preparation) and route of administration; the dosing rate; and the ability of the drug to gain access to the infection site. The chemical nature and physicochemical properties (in particular lipid solubility and degree of ionization) of the drug influence the extent of absorption (systemic availability), pattern of distribution, and rate of elimination (pharmacokinetic characteristics). The location of the infection can have a major influence on the drug concentration achieved where its action is required, as some sites (e.g., central nervous system) are protected by cellular barriers to drug penetration, while others (e.g., mammary glands) have a local pH that may favor drug accumulation (systemically administered lipid-soluble organic bases) but could alter antimicrobial activity. The urinary tract is unique in that very high concentrations may be attained in urine, particularly of antimicrobial agents that are mainly eliminated by renal excretion. Microbial susceptibility to the drug concentration achieved at the site of infection is critical in determining the clinical response to therapy (Chapter 2). Thus effective antimicrobial therapy depends on a triad of bacterial susceptibility, pharmacokinetic characteristics of the drug, and the dosage regimen. In addition, the competence of host defense mechanisms influences therapeutic outcome.

Routes of Administration

Drugs are administered as prepared dosage forms, including injectables, tablets, capsules, suspensions, oral pastes, and topicals. It is highly important that drug preparations be administered only by the route(s) and to the animal species for which their formulation was developed; this information is provided on the label of authorized products. When veterinary preparations of an antimicrobial agent are not available, preparations intended for use in humans are sometimes administered to companion animals. Knowledge of the fate of the drug is important, since dosage must be appropriate for the animal species.

Parenteral therapy should always be used in the treatment of severe infections, and in horses and ruminant species, it is generally preferable to oral therapy. Long-acting parenteral preparations should always be administered by IM injection and, apart from procaine penicillin G, are suitable for use only in ruminant species and pigs. In mild-to-moderate infections, oral therapy is preferred in dogs and cats, particularly for antimicrobial agents that are reliably absorbed from the gastrointestinal tract and those for which parenteral preparations cause tissue irritation at the IM site of injection. In the treatment of systemic infections caused by susceptible Gram-negative aerobic bacteria, aminoglycosides (such as gentamicin, amikacin) must be administered parenterally (generally IM or SC). Parenteral cephalosporins, with the notable exception of ceftiofur which is given by IM injection, should be administered by slow IV injection. Certain antimicrobials are approved for administra-

tion in the feed or drinking water to pigs or poultry, providing convenience of administration.

Intravenous Administration

The IV injection of a parenteral drug solution ensures that the total dose enters the systemic circulation. The high concentration initially produced in the blood declines rapidly as the drug distributes to other tissues of the body including the organs of elimination (liver and kidneys). Since passive diffusion is the process by which drug molecules enter cells and penetrate cellular barriers, the chemical nature of a drug, the lipid solubility and degree of ionization of those that are weak organic acids or bases, and the concentration gradient are the factors that determine the concentrations attained in cells, transcellular fluids (e.g., cerebrospinal, synovial and ocular), and glandular secretions (e.g., milk, saliva, prostatic fluid). After the attainment of pseudodistribution equilibrium, the plasma concentrations decline at a slower rate that is associated entirely with elimination (i.e., metabolism and excretion) of the drug. It is on the elimination phase of drug disposition that the half-life of the drug is based (Figure 4.1).

The IV administration of a parenteral solution assures complete systemic availability of the drug. Intravenous injection provides higher plasma concentrations which may enhance tissue distribution, but effective plasma concentrations generally persist for a shorter duration than following extravascular drug administration. A shorter dosage interval is required to maintain effective concentrations and the concentrations achieved will fluctuate to a greater degree. Parenteral solutions contain a drug in salt form dissolved in a vehicle, and the pH reaction of some solutions is far outside the physiologic range. To avoid excessively high initial drug concentrations in the systemic circulation and adverse effects that could be produced by the drug *per se* or by constituents of the formulation, IV injections should be given slowly. Parenteral solutions (conventional formulation only) that would produce tissue irritation at IM injection sites may be administered IV, but care must be taken to avoid perivascular damage. Pharmacokinetic parameters describing the disposition of a drug are based on the plasma concentration-time data following the IV injection of a single dose.

IV infusion of a parenteral solution containing a fixed concentration of drug is the only method of ad-

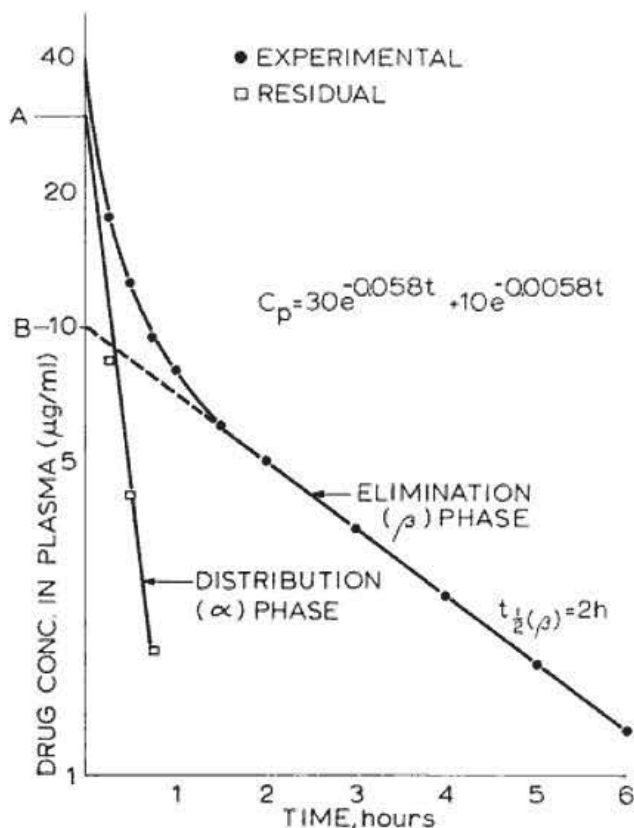


Figure 4.1. Plasma concentration-time curve for a drug after IV bolus injection. The disposition curve is described by a biexponential equation (inset) and separated into its component phases (distribution and elimination). The half-life of the drug is obtained from the exponent of the elimination phase ($\beta = 0.0058 \text{ min}^{-1}$; $t_{1/2} = 0.693/0.0058 = 120 \text{ minutes}$). From Baggot, 1977, with permission.

ministration that allows precise control over the rate of drug entry into the systemic circulation and the plasma concentration that will ultimately be attained. Assuming knowledge of the systemic clearance of the drug, this method can be used to achieve and maintain a desired steady-state concentration and avoid fluctuation in concentrations which is a feature of multiple dosing. While the rate of infusion determines the steady-state concentration attained, the time taken to reach steady-state is determined solely by the rate of elimination (half-life) of the drug. For practical purposes it can be predicted that a plasma concentration within 90 percent of the desired steady-state concen-

tration will be achieved after infusing the drug at a constant rate for a period corresponding to four times the half-life. It follows that the use of continuous infusion is most suitable for drugs with short half-lives (< 2 h). Should a change from one steady-state concentration to another be contemplated, infusion at a different rate for a similar length of time (i.e., 4 half-lives) will be required to effect the change in steady-state concentration.

Intramuscular and Subcutaneous Injections

Parenteral dosage forms (solutions and suspensions) of most antimicrobial agents can, in general, be administered by IM or SC injection to animals. The composition of the formulation, the concentration of the drug in the preparation, and the total dose to be administered will determine suitability of the dosage form for administration to a particular species. With regard to species, particular attention must be given to the concentration of drug in the preparation since drug concentration, together with the total dose required, determine the volume to be administered. A volume exceeding 20 mL should not be administered at any one IM injection site. The lateral neck is the preferred site for IM injection in large animals. While non-irritating parenteral solutions are frequently administered by SC injection in cats, this route of administration is seldom used in horses. Most antimicrobial agents are rapidly and completely absorbed from non-irritating solutions; peak plasma concentrations are reached within 1 hour of giving the injection. Although drug absorption from IM injection sites is generally assumed to be a first-order process, the validity of this assumption is often questionable. Oil-based formulations and unbuffered aqueous solutions or suspensions may cause irritation and produce tissue damage at IM injection sites. Slow, erratic absorption can result, leading to incomplete systemic availability of the drug.

Absorption from IM and SC injection sites is determined by the formulation of the parenteral preparation, the vascularity of the injection site and, to a lesser extent, the chemical nature and physicochemical properties of the drug substance. When single doses (10 mg/kg) of amikacin were administered SC to dogs at three different concentrations (50, 100, and 250 mg/mL), the concentration of the solution did not influence the absorption and elimination kinetics of the drug. The bioavailability of gentamicin (50 mg/mL)

was not affected by the location of the injection site (Gilman et al., 1987; Wilson et al., 1989). Comparison of the plasma concentration-time curves after IM injection in calves of five different parenteral preparations of ampicillin at similar dose levels (7.7 ± 1.0 mg/kg) shows the marked influence of formulation on the pattern of ampicillin absorption (Nouws et al., 1982) (Figure 4.2). Only drug preparations that are bioequivalent in the target animal species would be expected to have similar clinical efficacy.

The concentration of drug in a parenteral suspension can influence the plasma concentration profile. For example, when an aqueous suspension of amoxicillin trihydrate was administered IM to horses at the same dose level (10 mg/kg) but at different concentrations (100 and 200 mg/mL), the lower concentration (10%) was better absorbed and produced a more consistent plasma concentration profile. While the commercially available aqueous suspension of amoxicillin trihydrate may be administered by IM injection to cattle, it is unsuitable for clinical use in horses because of tissue irritation caused at the injection site.

Location of the injection site may also affect the systemic availability and peak plasma concentration of drugs administered as prolonged-release parenteral preparations. This was shown in a study of the influence of injection site location on the plasma concentration-time curves for penicillin G administered as procaine penicillin G to horses (Firth et al., 1986) (Figure 4.3).

The systemic availability and peak plasma concentration of penicillin G were highest following IM injection of the drug product in the neck region (*M. serratus ventralis cervicis*). This site was followed, in descending order, by *M. biceps* $>$ *M. pectoralis* $>$ *M. gluteus* or subcutaneously in the cranial part of the pectoral region. It appears that tissue irritation caused by some parenteral preparations is more severe after subcutaneous than intramuscular injection (Nouws and Vree, 1983; Korsrud et al., 1993). The systemic availability of amoxicillin, administered as amoxicillin trihydrate 20% aqueous suspension, was shown in dairy cows to vary as widely with IM injection site as between IM and SC sites (Rutgers et al., 1980). Based on this study and others in which the conventional formulation of oxytetracycline was administered IM at different sites, it can be concluded that the shoulder and neck regions for IM injection are superior to the buttock and to subcutaneous injection in cattle

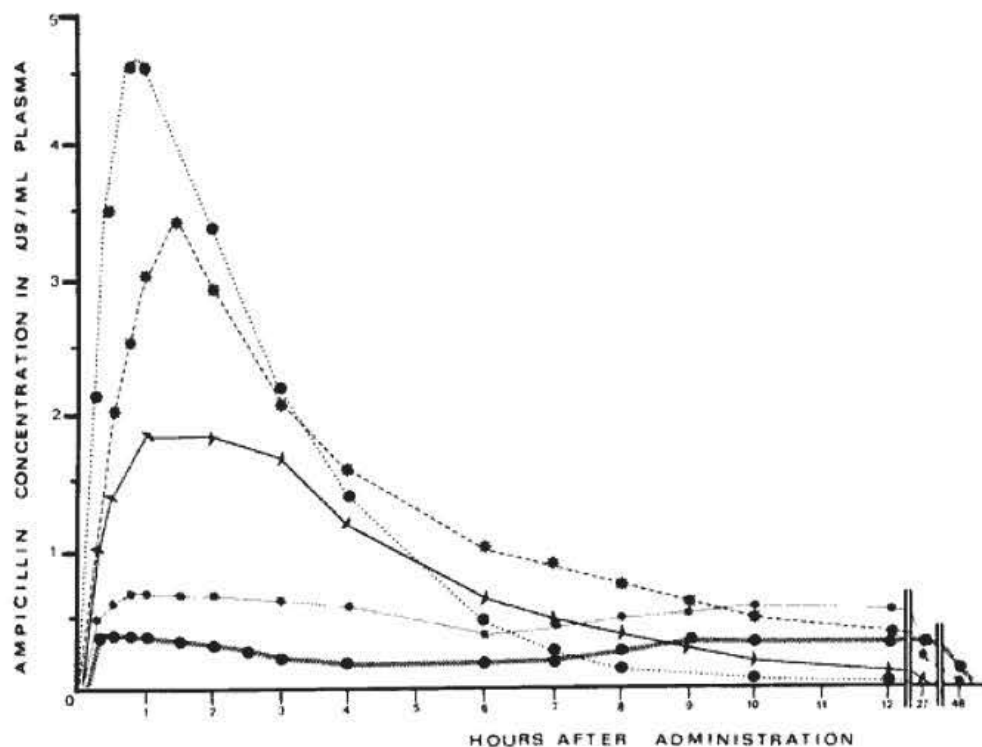


Figure 4.2. Mean plasma ampicillin concentrations after IM injection of 5 different parenteral ampicillin formulations at similar dose levels (7.7 ± 1.0 mg/kg) to 5 calves. From Nouws et al., 1982, with permission.

(Nouws and Vree, 1983). Better antimicrobial absorption from the former sites could be attributed to greater access of drug to a larger absorptive surface area with perhaps greater blood flow. Age or body weight of calves influenced the relative systemic availability, based on comparison of area under the curve, of amoxicillin (7 mg/kg) administered IM as amoxicillin trihydrate 10% aqueous suspension (Marshall and Palmer, 1980) (Figure 4.4). When the same preparation was administered IM to different animal species, the trend was for smaller animals (piglets, dogs, cats) to show an early high peak concentration followed by a rapid decline, while larger animals (calves, horses) showed a lower and relatively constant plasma concentration of amoxicillin over at least an 8-hour period.

Prolonged-release (long-acting) preparations are designed to delay absorption and thereby maintain effective drug concentrations for an extended period, which infers several times the elimination half-life of the drug. The aqueous suspension of procaine penicillin G (300,000 IU/mL) probably represents the limit to which decreasing the rate of absorption can be use-

fully applied to lengthen the dosage interval for penicillin G. A single dose (25,000 IU/kg) of this preparation will maintain effective concentrations against susceptible bacteria for at least 12 hours, and generally for 24 hours. An essential feature of prolonged-release preparations is that the rate of drug release be adequate to maintain effective plasma concentrations for the duration of the dosage interval.

A single IM dose (20 mg/kg) of a long-acting preparation of oxytetracycline base in 2-pyrrolidone provided plasma oxytetracycline concentrations greater than 0.5 µg/mL for 48 hours in ruminant calves, cattle, goats, red deer and fallow deer. Pronounced tissue damage at the injection site was found on examination of excised muscle tissue of pigs slaughtered 1 and 2 weeks after IM administration of the long-acting preparation, whereas the conventional preparation, administered at the same dose level (20 mg/kg), produced little tissue irritation (Xia et al., 1983). Comparison of the pharmacokinetics of three injectable oxytetracycline preparations administered IM in the lateral neck of pigs (20 mg/kg) indicates that 48 hours would be an appropriate dosage interval for either of

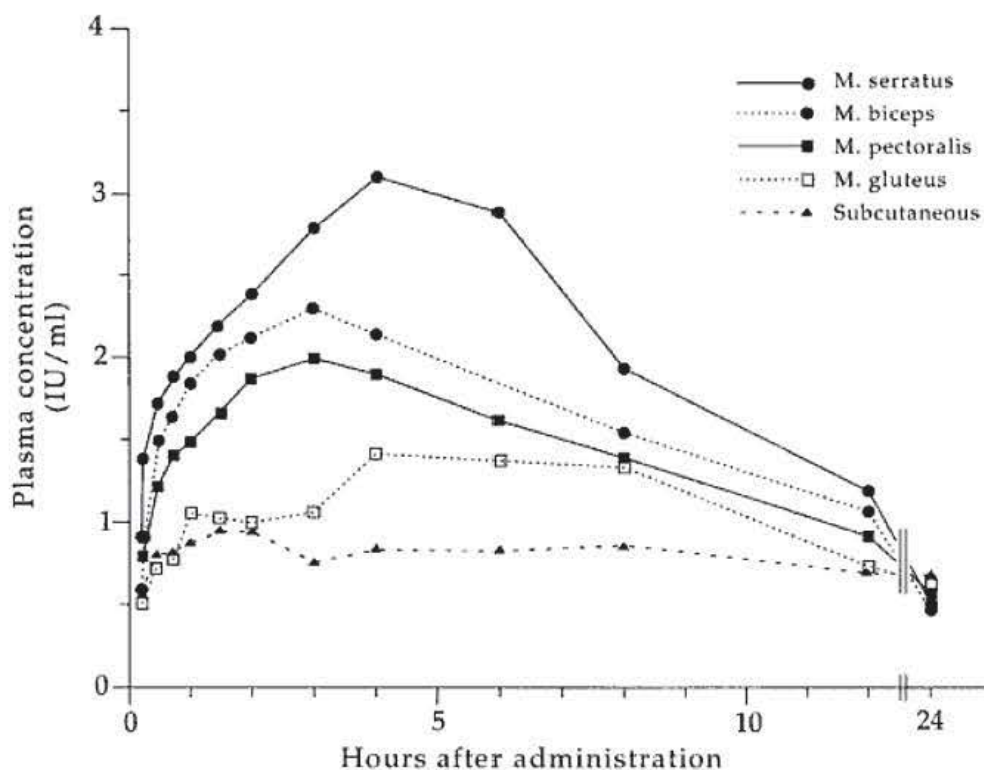


Figure 4.3. Mean plasma penicillin concentration-time curves after 20,000 IU of procaine penicillin G/kg was administered to 5 horses at 5 different sites. From Firth et al., 1986, with permission.

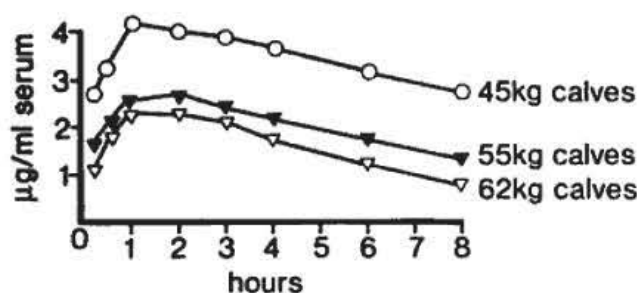


Figure 4.4. Effect of age and weight on systemic availability of amoxicillin in calves after IM injection of amoxicillin trihydrate aqueous suspension (100 mg/ml) at a dosage of 7 mg/kg body weight. From Marshall and Palmer, 1980, with permission.

the long-acting preparations and 24 hours for the conventional preparation (Banting and Baggot, 1996) (Table 4.1). Oxytetracycline formulated in polyethylene glycol may be the only long-acting oxytetracycline preparation that is suitable for IM administration in horses (Dowling and Russell, 2000).

Useful methods of evaluating the extent of tissue irritation and rate of resolution at the IM injection site

include the use of ultrasonography (Banting and Tranquart, 1991) and determination of the kinetics of plasma creatine kinase (CK) activity (Aktas et al., 1995; Toutain et al., 1995). The use of a tissue-damaging drug preparation in food-producing animals must entail a correspondingly long withdrawal period. The withdrawal period for a drug varies with formulation of the dosage form (preparation) and may differ between animal species. Parenteral preparations should be formulated in a manner such that their IM injection does not cause tissue damage with persistence of drug residues at the injection site. Irritating preparations and drugs in oil-based vehicles should *never* be administered to horses. With the notable exceptions of procaine penicillin G (aqueous suspension) and, when specifically indicated, oxytetracycline formulated in polyethylene glycol, long-acting parenteral preparations currently available are unsuitable for use in the horse.

Since avian and reptilian species appear to have a well developed renal portal system, first-pass renal excretion may decrease the systemic availability of drugs, especially those which undergo proximal tubular secretion such as beta-lactam antibiotics, injected IM in

Table 4.1. Pharmacokinetic parameters describing the absorption and disposition of three oxytetracycline formulations administered intramuscularly (lateral neck) to pigs.

Pharmacokinetic term	Product A	Product B	Product C
C_{max} ($\mu\text{g/ml}$)	6.27 ± 1.47	5.77 ± 1.0	4.68 ± 0.61
t_{max} (h)	3.0 (2.0–4.0)	0.5 (0.083–2.0)	0.5 (0.083–2.0)
AUC ($\mu\text{g} \cdot \text{h/ml}$)	79.22 ± 25.02	91.53 ± 20.84	86.64 ± 14.21
MRT (h)	11.48 ± 2.01	25.27 ± 9.22	37.66 ± 15.62
$C_{p(24h)}$ ($\mu\text{g/ml}$)	0.81 ± 0.34	1.01 ± 0.26	0.97 ± 0.29
$C_{p(48h)}$ ($\mu\text{g/ml}$)	<LOQ	0.40 ± 0.17	0.50 ± 0.09

Note: $n = 8$; dose = 20 mg/kg body weight. Results are expressed as mean \pm standard deviation. LOQ = limit of quantification (0.1 $\mu\text{g/ml}$). Product A: engemycin 10% in polyvinylpyrrolidone; product B: Oxyter LA 20% in dimethylacetamide; product C: terramycin LA 20% in 2-pyrrolidone and polyvinylpyrrolidone.

Source: Banting and Baggot (1996) with permission.

the legs (thighs) of birds or the posterior half of the body of reptiles.

Oral Administration

There are a wide variety of oral dosage forms available for use in animals. They include oral solutions, suspensions, pastes, capsules, tablets of various types and powders. The rate of drug absorption varies with the dosage form; oral solutions provide rapid absorption. Dissolution must precede absorption from a solid dosage form and frequently controls the rate of drug absorption. Oral suspensions and pastes generally provide drugs for absorption at a rate that is intermediate between solutions and solid dosage forms. Reticular groove closure may enable drug solutions to bypass the rumen, while drug suspensions are largely deposited in the rumen. This distinction may be of significance with regard to the clinical efficacy of some anthelmintics. Although the rumen has good absorptive capacity, drug absorption takes place slowly from ruminal fluid (pH 5.5–6.5) because of its large volume and slow onward passage to the abomasum. In monogastric species, gastric emptying is the principal physiologic factor governing the rate of drug absorption. Medication of feed or of drinking water provides a convenient means of antimicrobial administration to pigs and poultry. By contrast, the addition of an antimicrobial agent to the feed is an unreliable method of dosing horses and should not be considered.

The systemic availability, which is the fraction of an oral dose that reaches the systemic circulation unchanged, is of greater clinical importance than the rate of absorption of an antimicrobial agent. Systemic availability is influenced by the stability of an antimicrobial

agent in the highly acidic gastric contents (pH 3–4) or its susceptibility to inactivation (by hydrolytic or reductive reaction) by ruminal micro-organisms, and by the chemical nature and physicochemical properties of the drug. Since absorption takes place by passive diffusion across the mucosal epithelial barrier, high solubility in lipid is an important property. Having passed through the mucosal barrier, drug molecules are conveyed in hepatic portal venous blood to the liver, the major organ of drug metabolism, prior to reaching the systemic (general) circulation. Presystemic metabolism, referred to as the first-pass effect, can occur in the gut lumen or mucosal epithelium or, most importantly, in the liver. The first-pass effect decreases the systemic availability of drugs that undergo extensive hepatic metabolism. Presystemic metabolism activates prodrugs of ampicillin, such as pivampicillin and bacampicillin, by hydrolysis of the ester in the intestinal mucosa. Metabolic conversion (N-dealkylation) of enrofloxacin to ciprofloxacin and of difloxacin to sarafloxacin is likely to occur to some extent, but the products formed possess high antimicrobial activity, being drugs in their own right.

The systemic availability of aminoglycoside antibiotics, which are polar organic bases, is very low following oral administration, whereas they are rapidly absorbed and completely available systemically when administered by IM or SC injection. It is the absorption process that differs between the gastrointestinal tract and parenteral sites. Passage across the mucosal barrier requires that the drug be at least moderately lipid-soluble, but absorption from parenteral sites is mainly controlled by capillary blood flow at the absorptive surface.

The presence of food in the stomach or binding to feed constituents decreases the systemic availability of most penicillins, apart from amoxicillin and ampicillin prodrugs, oral cephalosporins, trimethoprim-sulphonamide combinations and tetracyclines (except doxycycline). The systemic availability of some drugs (e.g., doxycycline, erythromycin estolate, ketoconazole) is increased when administered to dogs after feeding. Since the systemic availability of antimicrobial agents administered orally (pastes) or by nasogastric tube (aqueous suspensions) to horses is significantly decreased by feeding prior to dosing, food should be withheld for up to 2 hours after antimicrobial administration. The systemic availability of metronidazole varies widely between individual horses (60–90%), but might not be significantly decreased by feeding prior to oral dosing (Baggot et al., 1988a).

It may be feasible to administer certain antimicrobial agents orally to young foals, calves and kids, even though these drugs are not suitable for oral use in older and adult herbivorous animals. This is not only due to better absorption, but to the fact that neither the microflora indigenous to the specialized fermentation regions of the gastrointestinal tract nor the hepatic microsomal oxidative reactions have developed.

Applied Clinical Pharmacokinetics

The chemical nature and related physicochemical properties largely govern the absorption, distribution and elimination, which refers to biotransformation (metabolism) and excretion, of antimicrobial agents. The majority of antimicrobial agents are weak organic electrolytes, either weak acids or weak bases, while fluoroquinolones, tetracyclines and rifampin are amphoteric compounds. Lipid solubility and the degree of ionization, which is determined by the pK_a of the drug and the pH of the biologic fluid in question (pH of blood is 7.4), influence the extent of absorption, the pattern of distribution, and the elimination process(es) for antimicrobial agents. Lipid solubility is a requirement for passive diffusion of drugs across cell membranes, and it is the nonionized form of weak organic acids and bases that is lipid-soluble.

Since antimicrobial agents, like other drugs, are available as prepared dosage forms, the type and formulation of the dosage form (drug preparation) de-

termine the route of administration, the bioavailability and overall rate of elimination of the drug. Because it affects pharmacokinetic processes, the drug preparation influences the dosage regimen for each animal species and the withdrawal period(s) in food-producing animals.

Distribution and Elimination

Following the entry of an antimicrobial agent into the systemic circulation, the free (unbound) fraction is available for distribution to extravascular tissues and for removal from the body by the organs of elimination (liver and kidneys). The extent and pattern of distribution vary between antimicrobial agents of different classes due to differences in their chemical nature. Distribution is determined by blood flow to tissues and the ability of a drug to penetrate (mainly by passive diffusion) cellular barriers. The rate of distribution is largely influenced either by perfusion (lipophilic drugs) or by diffusion (ionized and polar compounds). Extensive (> 80%) binding to plasma proteins limits the immediate availability of a drug for extravascular distribution. Accumulation in tissues (pH partition effect) influences the extent of distribution. Selective binding to a tissue component (e.g., aminoglycosides to phospholipid-rich tissues of the inner ear and kidney cortex) may account for only a small fraction of the amount of drug in the body but could produce an adverse, even toxic, effect or the residue could limit the use of the drug in food-producing animals. Definitive information on the distribution pattern of a drug can only be obtained by measuring levels of the drug in the various organs and tissues of the body, such as kidneys, liver, skeletal muscle, adipose tissue and skin. Selective binding can reasonably be suspected and should be further investigated when a specific lesion is produced in a tissue or terminal elimination is prolonged.

While some antimicrobial agents are almost entirely eliminated by renal excretion (aminoglycoside, most beta-lactam antibiotics), others are eliminated by hepatic metabolism and, to a lesser extent, by renal or biliary excretion. The extent to which liver damage decreases the rate of elimination of drugs is difficult to assess. However, certain antimicrobial agents (chloramphenicol, erythromycin, tiamulin, ketoconazole) inhibit hepatic microsomal enzyme activity, while rifampin and griseofulvin induce hepatic microsomal enzymes by increasing their synthesis. The rate of

elimination of several therapeutic agents used concurrently with one of these antimicrobials can be affected by the altered microsomal-mediated oxidative reactions. Metronidazole inhibits aldehyde dehydrogenase and thereby produces a disulfiram-like effect in human beings. Decreased renal function requires adjustment of aminoglycoside dosage (see below). Renal impairment may lead to the accumulation of drug metabolites although formed in the liver or at other sites of biotransformation.

Lipophilic antimicrobial agents readily penetrate cellular barriers, with the exception of the blood-brain barrier. Consequently, these drugs are well absorbed from the gastrointestinal tract, become widely distributed in body fluids and tissues, and can generally attain effective concentrations at sites of infection. Examples of lipophilic antimicrobial agents include fluoroquinolones, macrolides and lincosamides, minocycline and doxycycline, trimethoprim, rifampin, metronidazole and chloramphenicol. Some of these drugs (erythromycin, clindamycin, doxycycline) bind extensively to plasma proteins, which limits their availability for extravascular distribution. Clindamycin, however, may attain effective concentrations in bone. Of the lipophilic antimicrobials, only certain individual drugs penetrate the blood-brain and blood-CSF barriers and attain effective concentrations in cerebrospinal fluid (e.g., trimethoprim, metronidazole, chloramphenicol). In the presence of meningitis, most intravenously administered third-generation cephalosporins (except cefoperazone) penetrate the blood-CSF barrier. Fluconazole may be the only azole antifungal drug that penetrates the blood-brain barrier. Individual tetracyclines differ in lipid solubility which influences the tissue concentrations attained and their clinical efficiency.

Lipophilic antimicrobial agents are eliminated mainly by the liver (metabolism and biliary excretion), while a fraction of most of these drugs (with the notable exception of doxycycline) is excreted unchanged (and as metabolites) in the urine. The more rapidly a drug is metabolized, the smaller the fraction of dose that is excreted unchanged, e.g., trimethoprim (Table 4.2). The metabolic pathways, various hepatic microsomal oxidative reactions and glucuronide conjugation, are determined by the functional groups present in the drug molecule. Apart from some fluoroquinolones, rifampin, and metronidazole, the metabolites of lipophilic antimicrobials are inactive. Enroflox-

Table 4.2. Half-life and urinary excretion of trimethoprim.

Species	Half-life (h)	Fraction of Dose Excreted Unchanged (%)
Goat	0.7	2
Cow	1.25	3
Pig	2.0	16
Horse	3.2	10
Dog	4.6	20
Human	10.6	69 ± 17

acin is converted to ciprofloxacin, difloxacin to sarafloxacin, and pefloxacin to norfloxacin by N-dealkylation (oxidative reaction). The half-lives of individual lipophilic antimicrobials may differ within a species and between animal species. For example, the half-lives of various fluoroquinolones in the dog are: ciprofloxacin (2.2 h), enrofloxacin (3.4 h), norfloxacin (3.6 h), difloxacin (8.2 h) and marbofloxacin (12.4 h). The half-lives of metronidazole in various species are: cattle (2.8 h), horse (3.9 h), dog (4.5 h), chicken (4.2 h) and of chloramphenicol are: horse (0.9 h), dog (4.2 h), cat (5.1 h), chicken (5.2 h).

The pharmacokinetic properties of different antibacterial drug classes and their members, and factors affecting these properties, are discussed extensively under the description of each drug.

Pharmacokinetic Parameters

Drug disposition is the term used to describe the simultaneous effects of distribution and elimination, that is, the processes that occur subsequent to the absorption of a drug into the systemic circulation. The major pharmacokinetic parameters that describe the disposition of a drug are the systemic (body) clearance (Cl_B), which measures the ability of the body to eliminate the drug, and the volume of distribution (V_d), which denotes the apparent space in the body available to contain the drug. The half-life ($t_{1/2}$) expresses the overall rate of drug elimination; it is only when the dose is administered intravenously that the "true" (elimination) half-life of a drug can be determined. When a drug preparation is administered orally or by a non-vascular parenteral route (e.g., IM or SC), the systemic availability (F)—that fraction of the dose that reaches the systemic circulation unchanged—is an important parameter. Since the absorption process influ-

ences the rate of drug elimination, the value obtained for half-life is "apparent"; it will vary with route of administration and formulation of the dosage form (drug preparation).

Bioavailability, which refers to both the rate and extent of drug absorption, provides a more complete description of the absorption process. The rate and pattern of absorption assume importance when a drug is administered as a prolonged-release (long-acting) preparation.

Bioavailability

Bioavailability is defined as the rate and extent to which a drug enters the systemic circulation unchanged. It is influenced not only by the factors that determine drug absorption but also by formulation of the dosage form and the route of administration. Complete systemic availability (extent of absorption) can be assumed only when a drug is administered intravenously.

An estimation of the rate of drug absorption can be obtained from the peak (maximum) plasma concentration (C_{\max}) and the time at which the peak concentration is attained (t_{\max}), based on the measured (observed) plasma concentration-time data. However, the blood sampling times determine how well the peak is defined; t_{\max} often lies between measured plasma concentrations. Both C_{\max} and t_{\max} may be influenced by the rate of drug elimination, while C_{\max} is also affected by the extent of absorption. The parameter C_{\max}/AUC , which can be calculated and is expressed in units of reciprocal time (h^{-1}), is an additional term that may be used to indicate the rate of drug absorption. Even though an absorption rate constant (and half-life) can be calculated, the generally small number of data points on which it is based make it an inaccurate measurement of the rate of drug absorption. The usual technique for estimating systemic availability (F), a measure of the extent of absorption, employs the method of corresponding areas:

$$F = \frac{AUC_{PO}}{AUC_{IV}} \times \frac{Dose_{IV}}{Dose_{PO}}$$

where AUC is the total area under the plasma concentration-time curve relating to the route of drug administration (IV and PO, IM or SC). The application of this technique involves the assumption that clearance of the drug is not changed by the route of admin-

istration. Following the administration of a single dose by any route, the total area under the curve can be estimated by the linear trapezoidal rule, from time zero to the last measured plasma drug concentration, with extrapolation to infinite time, assuming log-linear decline (Figure 4.5). The accuracy of this method for estimating the total area under the curve (AUC) depends on the number of plasma concentration-time data points from the time of drug administration (time zero) to the last measured plasma concentration and on the relative area under the extrapolated portion of the curve, which should be less than 10% of the total area. When comparison is made of the AUC for an oral dosage form with that for an intravenous preparation of the drug, the absolute bioavailability (systemic availability) is obtained, whereas comparison of the AUCs for two oral dosage forms (test and reference) estimates the relative bioavailability. The latter comparison is used in bioequivalence assessment. In bioavailability studies a crossover design, with an appropriate washout period between the phases of the study, should be used whenever feasible.

The systemic availability of orally administered antimicrobial drugs is often incomplete (< 100%). This may be due to poor absorption, degradation in the stomach or rumen, or presystemic metabolism (first-pass effect). Incomplete systemic availability can often be compensated for by administering a higher oral dose. The time of feeding relative to oral dosing may affect the systemic availability (oral bioavailability) of an antimicrobial agent, as discussed earlier. For example, the oral bioavailability of enrofloxacin, trimethoprim and sulfadiazine is high (> 80%) in pigs and is not influenced by the intake of feed. In contrast, the presence of feed in the gastrointestinal tract markedly decreases the oral bioavailability of spiramycin (60 to 24%) and lincomycin (73 to 41%) in the pig (Nielsen, 1997). The systemic availability of rifampin (5 mg/kg) was 26% when the drug was administered to horses 1 h after feeding, compared with 68% when given 1 h before feeding (Figure 4.6). Because of species differences in digestive physiology and in anatomical arrangement of the gastrointestinal tract, the systemic availability and rate of absorption of drugs administered orally differ widely between ruminant and monogastric species.

Parenteral preparations administered by IM injection often vary in systemic availability, while the rate of absorption differs between conventional

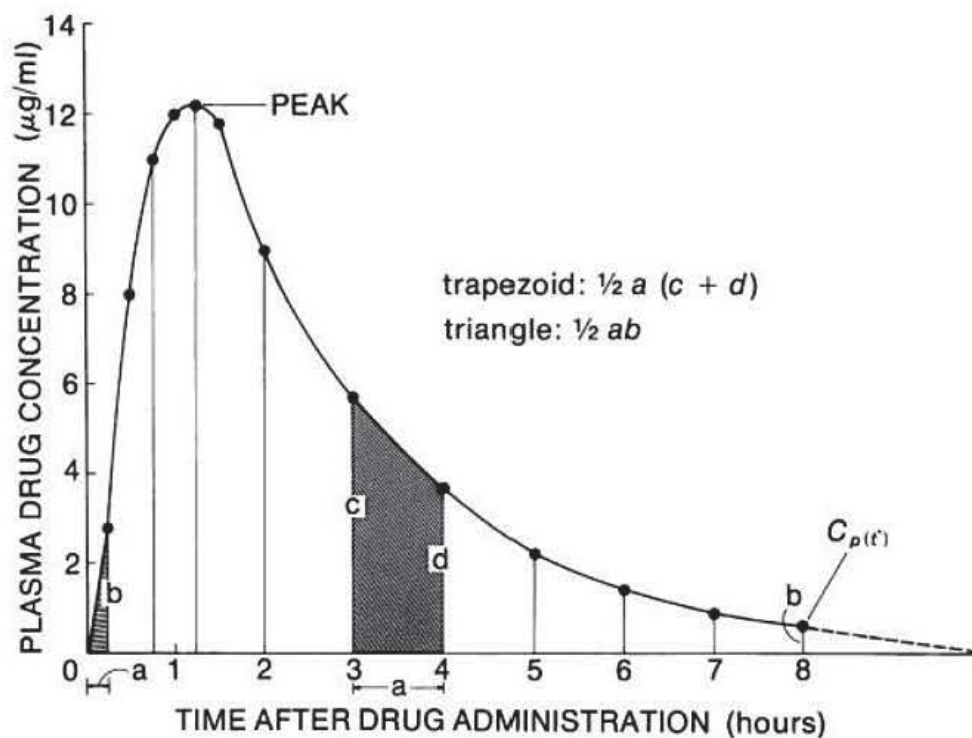


Figure 4.5. Typical plasma drug concentration profile following oral administration or nonvascular (IM, SC) injection of a conventional form of a drug. AUC may be calculated by the trapezoidal rule. From Baggot, 1977, with permission.

(immediate-release) and long-acting (prolonged-release) dosage forms. Incomplete systemic availability of parenteral preparations could be attributed either to partial precipitation of the drug at the injection site or to tissue irritation caused by the drug *per se*, the vehicle, or the pH of the preparation. By decreasing the rate of drug absorption, long-acting preparations provide a prolonged duration of effective plasma concentrations and allow the use of a longer dosage interval. For example, the dosage interval for procaine penicillin G is 12 h in horses, and 24 h in pigs and cattle; the dosage interval for the long-acting parenteral formulation of oxytetracycline is 48 h in pigs, cattle and goats. Repeated dosage with prolonged-release preparations produces less fluctuation in plasma concentrations than the degree of fluctuation produced by conventional preparations. It is usual to determine the relative bioavailability of a prolonged-release preparation by comparing area under the curve with AUC for a conventional preparation administered by the same route to the same animals (crossover design). The mean residence times should be compared. The plasma concentration-time curve, plotted on arithmetic coordinates, shows the pattern of drug absorp-

tion and the duration of effective plasma concentrations. It is on the latter, rather than the apparent half-life, that the dosage interval is based.

The systemic availability of a drug can be estimated by comparing the cumulative urinary excretion of the unchanged (parent) drug after extravascular administration with the amount excreted unchanged after IV administration. Using this approach, the systemic availability of oxytetracycline was determined in pigs following the IM injection (biceps femoris) of single doses (20 mg/kg) of a conventional (OTC-C) and a long-acting (OTC-LA) preparation (Figure 4.7). Both preparations provided over 95 percent systemic availability of the antibiotic (Xia et al., 1983). Cumulative urinary excretion was used to compare the systemic availability of sulfamethazine administered as three oral dosage forms to yearling cattle (Bevill et al., 1977). The results obtained (Table 4.3) indicate that the oral solution (107 mg/kg) and oral rapid-release bolus (27.8 g of sulfamethazine; similar dose level as for oral solution) provide relatively effective availability of the drug for absorption from the rumen, whereas the sustained-release bolus (67.5 g of sulfamethazine) is a less satisfactory dosage form. This

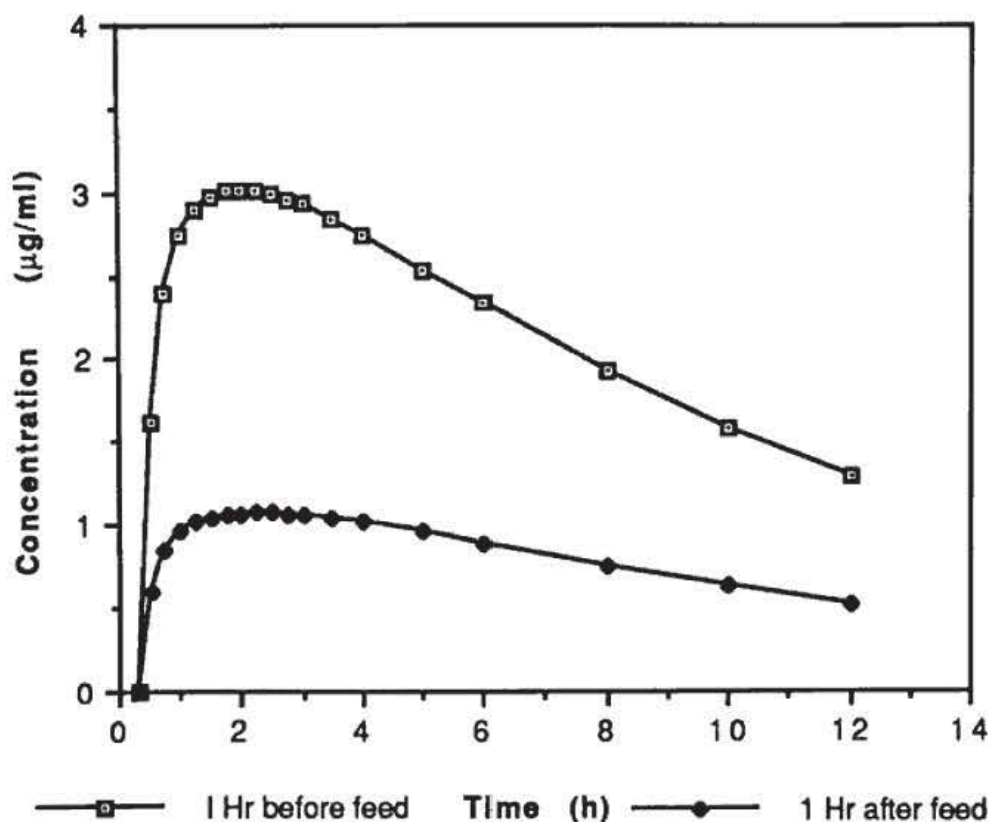


Figure 4.6. Mean plasma rifampin concentration curves in horses ($n=5$) after oral administration of the drug (5 mg/kg) 1 hour before or 1 hour after feeding.

method is an alternative to comparing area under the plasma concentration-time curves, but it is cumbersome to apply since the total volume of urine voided during the excretion period for the drug (at least 4 half-lives) must be measured. In addition, the stability of the drug in urine during the collection period and storage of the samples must be assured. Use of cumulative urinary excretion data to compare the systemic availability of different dosage forms of a drug administered by the same extravascular route (PO or IM), i.e., relative bioavailability, assumes that the ratio of the total amount excreted unchanged to the amount absorbed remains constant. It is always preferable to base estimation of the rate of drug absorption on plasma concentration data rather than on urinary excretion data.

Clearance

Clearance indicates the volume of blood or plasma from which a drug (or marker substance for an elimination process) would have to be cleared per unit of

Table 4.3. Systemic availability of three oral dosage forms of sulfamethazine in cattle.

Dosage Form	Systemic Availability (%)
Solution	80.8
Rapid-release bolus	63.2
Sustained-release bolus	32.0

time to account for its elimination. For comparative purposes, clearance is expressed in units of $\text{mL/min} \times \text{kg}$. When based on plasma drug concentrations, clearance can assume values that are not "physiologic"; conversion from plasma to blood clearance can be accomplished.

The systemic (body) clearance of a drug represents the sum of the clearances by the various organs (liver, kidneys, other organs or tissues) that contribute to elimination of the drug. It can be calculated by dividing the systemically available dose by the total area

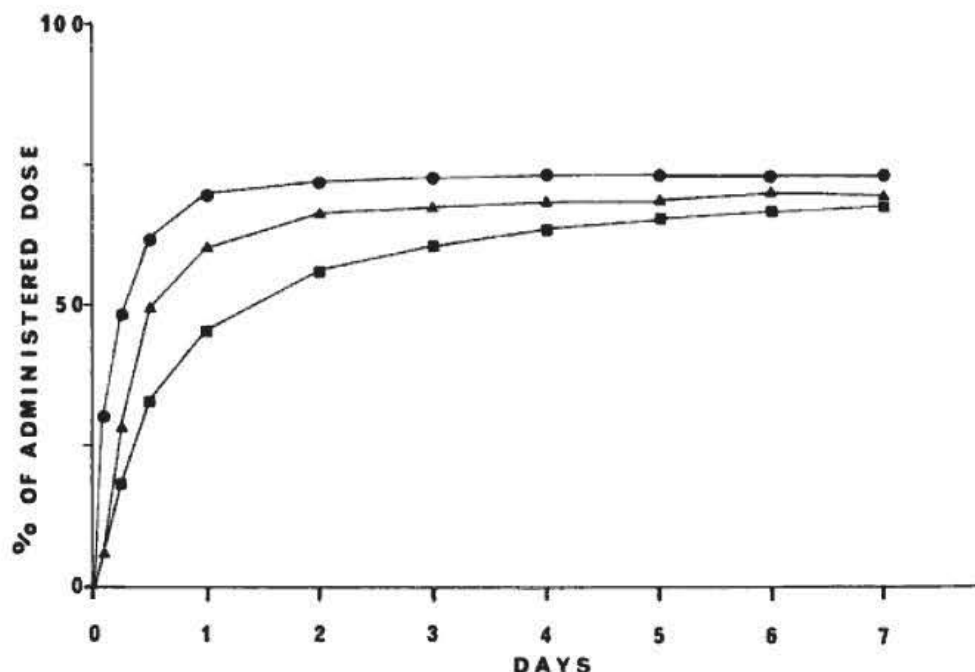


Figure 4.7. Cumulative urinary excretion of oxytetracycline in pigs after IV injection of conventional OTC preparations (●, $n=3$) and IM injection of conventional (▲, $n=4$) and a long-acting OTC preparation (■, $n=6$). From Xia et al., 1983, with permission.

under the plasma concentration-time curve (from time zero to infinity):

$$Cl_B = \frac{F \times \text{Dose}}{\text{AUC}}$$

where F is the fraction of the dose that enters the systemic circulation unchanged and AUC is the total area under the curve.

By definition, the systemic clearance of a drug is the product of the volume of distribution, calculated by the area method, and the overall elimination rate constant:

$$Cl_B = V_{d(\text{area})} \times \beta$$

When an intravenous dosage form of the drug is not available, F cannot be determined; in this situation the term Cl_B/F should be used.

The concept of clearance is extremely useful in clinical pharmacokinetics, since the systemic clearance of most therapeutic (including antimicrobial) agents is constant over the clinically useful range of plasma concentrations. This is because the overall elimination of most drugs obeys first-order kinetics, whereby a constant fraction is eliminated per unit of time (e.g.,

50% is eliminated each half-life). Systemic clearance is probably the most important pharmacokinetic parameter to consider in defining a drug dosage regimen and is required for calculating dosing rate adjustment that may be necessitated by functional impairment of an organ of elimination. When multiple doses are administered at a constant dosage interval, systemic clearance relates the average steady-state plasma concentration to the dosing rate of the drug. Systemic or individual organ clearance, depending on the elimination processes for a drug, may be the pharmacokinetic parameter of choice in applying the allometric technique to interspecies scaling of drug elimination. As an example of one species, values of pharmacokinetic parameters describing the disposition of some antimicrobial agents are presented for dogs (Table 4.4).

Volume of Distribution

The volume of distribution, which relates the amount of drug in the body to the concentration in the plasma, provides an estimation of the extent of distribution of a drug. It quantifies the apparent space, in both the systemic circulation and the tissues of distribution, available to contain the drug, but does not reveal the pattern of distribution. The distribution pattern of a

Table 4.4. Disposition kinetics of antimicrobial agents in dogs.

Drug	Half-life (h)	$V_d(\text{area})$ (ml/kg)	Cl_B (ml/min \times kg)
Penicillin G	0.50	156	3.60
Ampicillin	0.80	270	3.90
Ticarcillin	0.95	340	4.30
Cephalexin	1.71	402	2.70
Cefazolin	0.80	700	10.40
Cefotaxime	0.73	480	7.50
Ceftizoxime	1.07	300	3.25
Ceftazidime	0.82	220	3.15
Ceftriaxone	0.85	240	3.26
Gentamicin	1.25	335	3.10
Amikacin	1.10	245	2.61
Kanamycin	0.97	255	3.05
Norfloxacin	3.56	1,770	5.53
Enrofloxacin	3.35	2,454	8.56
Marbofloxacin	12.40	1,900	1.66
Difloxacin	8.20	3,640	5.10
Trimethoprim	4.63	1,849	4.77
Sulfadiazine	5.63	422	0.92
Sulfadimethoxine	13.20	410	0.36
Sulfisoxazole	4.50	300	0.77
Chloramphenicol	4.20	1,770	4.87
Thiamphenicol	1.75	765	5.20
Metronidazole	4.50	948	2.50
Erythromycin	1.72	2,700	18.2
Clindamycin	3.25	1,400	5.25
Oxytetracycline	6.02	2,096	4.03
Doxycycline	6.99	1,010	1.72
Minocycline	6.93	1,952	3.55

drug can only be described by measuring the level (amount) of drug in the various organs and tissues of the body.

The volume of distribution can be calculated (area method) from the equation:

$$V_{d(\text{area})} = \frac{\text{Dose}}{\text{AUC} \times \beta}$$

where AUC is the total area under the plasma concentration-time curve and β is the overall elimination rate constant of the drug, obtained from the linear terminal (elimination) phase of the semi-logarithmic disposition curve (Figure 4.1). This implies that the drug was administered as an IV bolus dose. When the drug is administered orally (PO) or by a nonvascular parenteral route (IM, SC), correction must be made for systemic availability (F) and the ap-

parent first-order elimination rate constant (k_d) be substituted for β .

Drugs that are predominantly ionized in plasma or are relatively polar (penicillins, cephalosporins, aminoglycosides) have volumes of distribution in the range 150 to 300 mL/kg; this infers no more than their distribution is limited in extent. Lipophilic antimicrobial agents (macrolides, lincosamides, chloramphenicol, trimethoprim, fluoroquinolones) have volumes of distribution that are generally between 1 and 3 L/kg. The volumes of distribution of moderately lipid-soluble antimicrobials (e.g., metronidazole, rifampin, sulfonamides) are intermediate (400 to 800 mL/kg). The tetracyclines differ in lipid solubility and their volumes of distribution vary accordingly.

Species variations in the volume of distribution of a drug can be largely attributed to differences in body composition (Table 4.5), in particular anatomical features of the gastro-intestinal tract, while differences in plasma protein binding may contribute. The greatest variation is found between ruminant and monogastric species, mainly for lipophilic organic bases.

Since volume of distribution, serving as a proportional factor, relates the plasma concentration to the amount of drug in the body, knowledge of this parameter is required for calculating the dose (mg/kg) that would provide a desired plasma drug concentration:

$$\text{Dose}_{iv} = C_{p(\text{ther})} \times V_{d(\text{area})}$$

Drug administration by the oral or a nonvascular parenteral route may require upward adjustment of the dose to compensate for incomplete systemic availability of the drug. No provision can be made for variation in the rate of drug absorption.

Volume of distribution has useful applications, but it is a parameter (volume term) that must be properly interpreted. Although $V_{d(\text{area})}$ may be determined following drug administration by any route, it varies with change in the elimination rate constant for a drug, even when the distribution space has remained unchanged. The volume of distribution at steady-state $V_{d(ss)}$ is not subject to this disadvantage, but can only be determined when the drug is administered as an IV bolus dose. The volume of distribution at steady-state can be calculated by the use of areas (Benet and Galeazzi, 1979):

$$V_{d(ss)} = \frac{\text{Dose}_{iv} \times \text{AUMC}}{(\text{AUC})^2}$$

Table 4.5. Body composition of various species (% live weight).

Organ/Tissue	Horse	Dog	Goat	Cow	Human
Blood	8.6	7.2	8.0	7.7	7.9
Brain	0.21	0.51	0.29	0.06	2.0
Heart	0.66	0.82	0.48	0.37	0.47
Lung	0.89	0.89	0.88	0.71	1.4
Liver	1.3	2.32	1.95	1.22	2.6
Spleen	1.11	0.26	0.25	0.16	0.26
Kidney	0.36	0.61	0.35	0.24	0.44
Gastrointestinal tract	5.8	3.9	6.4	3.8	1.7
Gastrointestinal contents	12.7	0.72	13.9	18.4	1.4
Skin	7.4	9.3	9.2	8.3	3.7
Muscle	40.1	54.5	45.5	38.5	40.0
Bone	14.6	8.7	6.3	12.7	14.0
Tendon	1.7	—	—	—	2.0
Adipose	5.1	—	—	18.9	18.1
Body weight (kg)	308	16	39	620	70
Source	a	b	b	c	d

Sources: a, Webb and Weaver (1979); b, Neff-Davis et al. (1975); c, Matthews et al. (1975); d, International Commission on Radiological Protection (1975).

where AUC is the total area under the curve (zero moment) and AUMC is the area under the first moment of the plasma concentration-time curve, that is, the area under the curve of the product of time and plasma concentration ($t \times C_p$) over the time span zero to infinity. This noncompartmental method of calculating V_d does not require the application of a compartmental pharmacokinetic model or mathematical description of the disposition curve. The volume of distribution at steady-state represents the volume in which a drug would appear to be distributed during steady-state if the drug existed throughout that volume at the same concentration as in the plasma.

The volume of distribution at steady-state is somewhat smaller than that calculated by the area method. Volumes of distribution of trimethoprim in dogs are $V_{d(ss)}$ 1675 mL/kg, $V_{d(area)}$ 1849 mL/kg, and of sulfadiazine are $V_{d(ss)}$ 392 mL/kg, $V_{d(area)}$ 422 mL/kg. When interpreting the influence of disease or physiologic state on the disposition kinetics of a drug, the systemic clearance (Cl_B) and $V_{d(ss)}$, rather than $V_{d(area)}$, are the pharmacokinetic parameters that should be used. Neither volume of distribution term allows one to predict drug concentrations that are attained in tissues or at infection sites.

Half-life

The half-life of a drug expresses the time required for the plasma concentration, as well as the amount in the

body, to decrease by 50% through the process of elimination. Half-life ($t_{1/2}$) measures the rate of decline in plasma drug concentrations during the elimination phase of the disposition curve, and is calculated from the expression:

$$t_{1/2} = \frac{0.693}{\beta}$$

where β is the overall elimination rate constant of the drug; 0.693 is $\ln 2$. The half-lives of antimicrobial agents are independent of the dose administered (at least within the recommended dose range), since their overall elimination obeys first-order kinetics. The characteristic of first-order elimination is that the time required for a given concentration to decrease by a certain fraction (e.g., 50% each half-life) is usually independent of the concentration.

Half-life is the pharmacokinetic parameter that is used to compare the rate of elimination of drugs in different species (Table 4.6). Even though the relative contribution of hepatic metabolism or renal excretion to antimicrobial elimination may differ between species, this approach is useful for comparative purposes. The half-lives of antimicrobials (and pharmacologic agents) that are mainly eliminated by hepatic metabolism can vary widely among species. Apart from oxytetracycline, which undergoes enterohepatic circulation, variation between mammalian species in the half-lives of antimicrobials that are eliminated by

Table 4.6. Average half-lives of antimicrobial agents in various species.

Drug	Process(es) of Elimination	Half-life (h)			
		Cattle	Horses	Dogs	Humans
Trimethoprim	M + E(r)	1.25	3.2	4.6	10.6
Sulfadiazine	M + E(r)	2.5	3.6	5.6	9.9
Sulfamethoxazole	M + E(r)	2.3	4.8	—	10.1
Sulfamethazine	M + E(r)	8.2	9.8	16.8	—
Sulfadimethoxine	M + E(r)	12.5	11.3	13.2	40
Sulfadoxine	M + E(r)	10.8	14.2	—	150
Norfloxacin	M + E(r)	2.4	6.4	3.6	5.0
Enrofloxacin	M + E(r)	1.7	5.0	3.4	—
Chloramphenicol	M + E(r)	3.6	0.9	4.2	4.6
Metronidazole	M + E(r)	2.8	3.9	4.5	8.5
Tinidazole	M + E(r)	2.4	5.2	4.4	14.0
Erythromycin	E(h) + M	3.2	1.0	1.7	1.6
Oxytetracycline	E(r ± h)	4.0	9.6	6.0	9.2
Penicillin G	E(r)	0.7	0.9	0.5	1.0
Ampicillin	E(r)	0.95	1.2	0.8	1.3
Cefazolin	E(r)	—	0.65	0.8	1.8
Ceftriaxone	E(r)	—	1.62	0.85	7.3 ^a
Gentamicin	E(r)	1.8	2.2–2.8	1.25	2.75
Amikacin	E(r)	—	1.7	1.1	2.3

Note: M, metabolism; E, excretion; r, renal; h, hepatic.

^aEliminated by the liver (biliary excretion) in human beings.

renal excretion is not of clinical significance. For comparative purposes the half-life of gentamicin which is eliminated by glomerular filtration, is about 1 hour in guinea pigs and rabbits, 1.1 to 1.4 h in dogs and cats, 1.4 to 1.8 h in cattle, sheep and goats, 1.9 h in pigs, 2 to 3 h in horses and human beings, *ca.* 3 h in llamas and camels, 2.5 to 3.5 h in chickens and turkeys, 12 h in channel catfish (*Ictalurus punctatus*) at $22 \pm 2^\circ\text{C}$, and an average of 51 h in reptiles.

Compared with mammalian (and avian) species, the half-lives of antimicrobials in poikilothermic species (fish and reptiles) are prolonged, which is consistent with their much lower metabolic turnover rate (Calder, 1984) (Chapters 38, 39). The half-life of an antimicrobial agent in fish is influenced by the temperature of the water in which the fish are acclimatized (Table 4.7). The overall rate of antimicrobial elimination increases (i.e., half-life decreases) with increase in water temperatures. The average half-life of trimethoprim, administered IV as trimethoprim-sulfadiazine combination, in carp (*Cyprinus carpio* L.) is 40.7 h at 10°C and 20 h at 24°C (Nouws et al., 1993) compared with cattle (1.25 h), horse (3.2 h), dog (4.6 h) and human beings (10.6 h). Sulfadiazine half-life similarly

differs widely: carp (47 h at 10°C ; 33 h at 24°C), cattle (2.5 h), horse (3.6 h), dog (5.6 h) and human being (9.9 h). The prolonged half-lives of lipid-soluble antimicrobials in fish could be attributed to a greater contribution made by enterohepatic circulation. The half-life of oxytetracycline in African catfish (*Clarias gariepinus*) is 80.3 h at 25°C and in rainbow trout (*Salmo gairdneri*) is 89.5 h at 12°C (Grondel et al., 1989), compared with half-lives in the range 3.4 to 9.6 h in domestic animals. When developing antimicrobial products for use in farmed fish, studies of the relationship between pharmacokinetics of the drugs and ambient (water) temperature should be performed (Chapter 39). Furthermore, the quantitative susceptibility (MIC) of bacterial pathogens isolated from poikilothermic animals may be temperature-dependent.

Half-life is the parameter on which selection of the dosage interval for a drug is based. The rate at which a drug administered by constant infusion or as multiple doses at a fixed interval (e.g., approximately equal to the half-life) approaches a steady-state concentration is determined solely by the half-life of the drug; a duration of four times the half-life is required to attain an average plasma concentration during the dosage interval

Table 4.7. The half-lives of various antimicrobial agents in fish.

Antimicrobial Agent	Species	Acclimatization Temperature (°C)	t _{1/2} (h)
Trimethoprim	Carp	10	40.7
	(<i>Cyprinus carpio</i> L.)	24	20.0
Sulfadiazine	Carp	10	47.0
	(<i>Cyprinus carpio</i> L.)	24	33.0
Oxytetracycline	Rainbow trout	12	89.5
	(<i>Salmo gairdneri</i>)		
	African catfish	25	80.3
	(<i>Clarias gariepinus</i>)		
Florfenicol	Atlantic salmon	10.8 ± 1.5	12.2
	(<i>Salmo salar</i>)	(seawater)	
Enrofloxacin	Fingerling rainbow trout	15	27.4
	(<i>Oncorhynchus mykiss</i>)		
Enrofloxacin (5 mg/kg, IM)	Red pacu	25	28.9
	(<i>Colossoma brachypomum</i>)		
Gentamicin	Channel catfish	22	12.0
	(<i>Ictalurus punctatus</i>)		
Sulfadimidine	Carp	10	50.3
	(<i>Cyprinus carpio</i> L.)	20	25.6
	Rainbow trout	10	20.6
	(<i>Salmo gairdneri</i>)	20	14.7

within 90% of the eventual steady-state concentration. A drug that selectively binds to tissues or is sequestered in a body compartment may have more than one half-life in any species. The relevance of the half-life chosen depends on the proposed application. The half-life based on the decline in plasma concentrations of clinical interest is relevant to dosage interval selection. That based on the gradual decline in sub-inhibitory plasma concentrations in the case of an antimicrobial agent may find application in predicting the withdrawal period for the drug in a food-producing species. The half-life of gentamicin (10 mg/kg, IV) in sheep based on the clinically relevant (β) elimination phase is 1.75 h, while that based on the prolonged terminal (γ) phase is 88.9 h (Brown et al., 1986). For drugs that show linear pharmacokinetic behavior (antimicrobial agents), dissimilar values of clearance based on single dose and average steady-state plasma concentrations (multiple dosing) provides definitive evidence of the presence of a “deep” peripheral compartment (Browne et al., 1990). Requirements of the study design are that the duration of blood sampling be prolonged and the sensitivity of the analytical method be sufficiently high to detect the presence of a deep peripheral compartment; the plasma concentration-time data is analyzed according to a three-compartment open model.

Mean residence time

The mean residence time (MRT) represents the average time the molecules of a drug reside in the body after the administration of a single dose. This parameter is the statistical moment analogy to half-life and may vary with the route of administration. The calculation of MRT is based on total areas under the plasma concentration curves, which are estimated by numerical integration using the trapezoidal rule (from time zero to the last measured plasma concentration) with extrapolation to infinite time:

$$\text{MRT} = \frac{\text{AUMC}}{\text{AUC}}$$

where AUC is area under the curve (zero moment) and AUMC is area under the (first) moment curve obtained from the product of plasma concentration and time *versus* time from time zero to infinity. The areas under the extrapolated portion of the curves are estimated by:

$$\frac{C_{p(\text{last})}}{\beta}$$

for AUC, and

$$\frac{t^* \times C_{p(\text{last})}}{\beta} + \frac{C_{p(\text{last})}}{\beta}$$

for AUMC, where β is the overall elimination rate constant of the drug and t^* is the time of the last measured plasma drug concentration ($C_{p(\text{last})}$). The elimination rate constant β is obtained by least squares regression analysis of the terminal 4 to 6 data points. It is desirable that the areas under the extrapolated portion of the curves be less than 10 per cent of the total AUC and less than 20 per cent of total AUMC.

Values of mean residence time and other pharmacokinetic parameters obtained for metronidazole in horses are presented (Table 4.8).

The advantage of using noncompartmental methods for calculating pharmacokinetic parameters, such as mean residence time (MRT), systemic clearance (Cl_B), volume of distribution ($V_{d(\text{area})}$), and systemic availability (F), are that they can be applied to any route of administration and do not require the selection of a compartmental model. The only assumption made is that the absorption and disposition of the drug obey first-order (linear) pharmacokinetics. After intravenous administration of a bolus dose of drug, the volume of distribution at steady-state is given by:

$$V_{d(ss)} = Cl_B \times MRT_{IV}$$

Changes in drug disposition

Certain physiologic conditions (neonatal period, pregnancy), prolonged fasting (48 h or longer), disease states (fever, dehydration, chronic liver disease, renal function impairment), or pharmacokinetic-based drug interactions may alter the disposition of drugs. Assessment of changes in the disposition of a drug should include a comparison of the plasma concentration-time curves in healthy and affected animals and of the following pharmacokinetic parameters: systemic clearance, volume of distribution at steady-state as well as that calculated by the area method, and the half-life of the drug.

The time course of a drug in the body depends upon both the volume of distribution and the systemic clearance, while half-life reflects the relationship between these two parameters:

$$t_{1/2} = \frac{0.693 \times V_{d(\text{area})}}{Cl_B}$$

Table 4.8. Bioavailability, absorption, and disposition kinetics of metronidazole after administration of single IV and oral doses to quarter horse mares.

Pharmacokinetic Terms and Units		Mean \pm s.d.
Intravenous		
$V_{d(\text{area})}$	(ml/kg)	661 \pm 44
$V_{d(st)}$	(ml/kg)	651 \pm 45
Cl_B	(ml/kg ? h)	115 \pm 10.8
$t_{1/2}$	(h)	4.04 \pm 0.45
MRT_{IV}	(h)	6.02 \pm 0.91
Oral		
Lag time	(h)	0.3 (0–0.88) ^a
t_{max}	(h)	1.5 (0.75–4.0) ^a
C_{max}	(μ g/ml)	21.2 \pm 3.1
$t_{1/2(d)}$	(h)	6.0 \pm 2.94
MRT_{PO}	(h)	9.4 \pm 4.32
F	(%)	74.5 \pm 13.0
		72.7 (58.4–91.5) ^a

Note: n=6; IV dose=10 mg/kg; oral dose=20 mg/kg.

^aMedian (range) of F ; note wide individual variation.

Source: Baggot et al. (1988a) with permission.

It follows that an alteration in either or both of the basic parameters, V_d and Cl_B , may result in a change in the half-life, which is a derived parameter. Because of the variables on which the half-life depends, it cannot be used as the sole pharmacokinetic parameter to interpret the underlying changes associated with altered disposition of a drug.

Changes in volume of distribution may occur in disease or physiologic states where membrane permeability is altered (fever), extracellular fluid volume is changed (dehydration, neonatal period), or drug binding to plasma proteins is decreased (hypoproteinemia, uremia, competitive drug displacement). In studies of the effect of *E. coli* endotoxin-induced fever in dogs and etiocholanolone-stimulated fever in human beings on the serum concentrations of gentamicin, it was shown that serum gentamicin concentrations were lower during the febrile state, while the renal clearance (gentamicin is eliminated entirely by glomerular filtration) and the half-life of the drug were not significantly changed (Pennington et al., 1975). The lower serum concentrations could be attributed to increased extravascular distribution, although not of sufficient extent to significantly increase the half-life, of the aminoglycoside. Penicillin G dis-

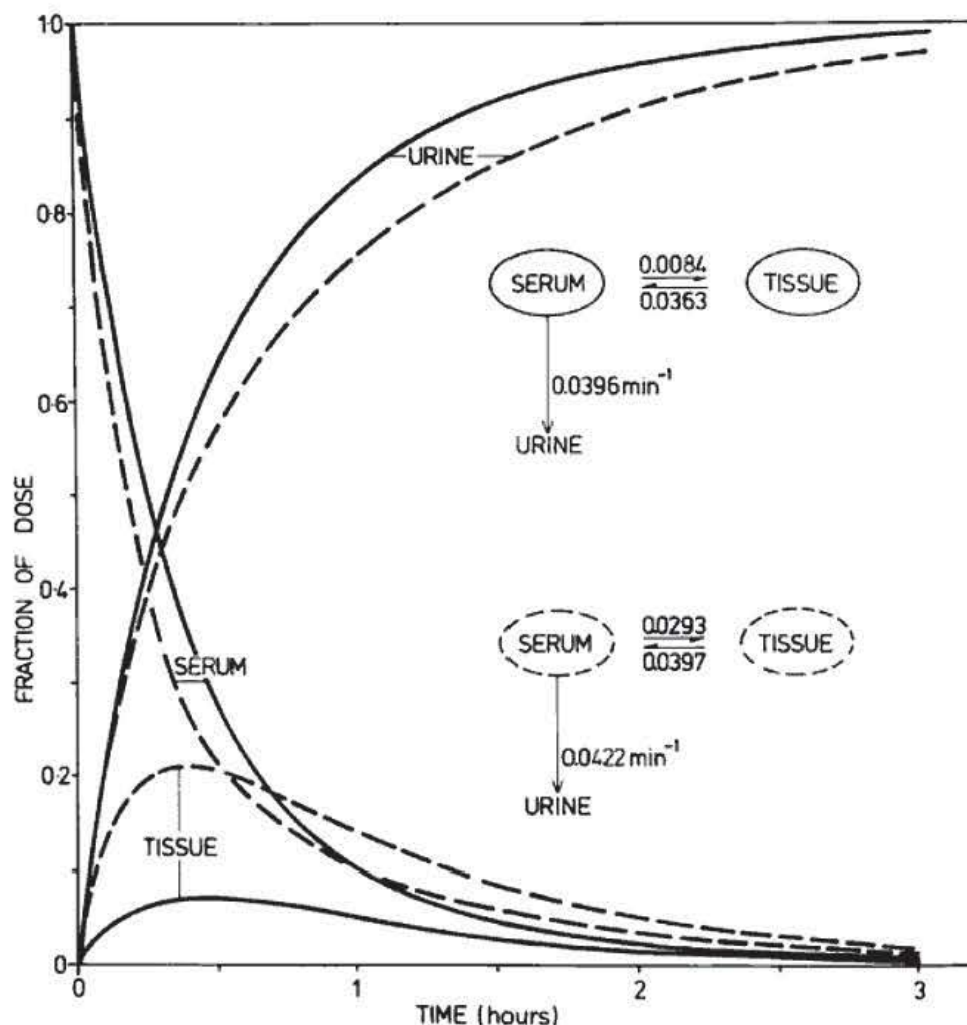


Figure 4.8. Analog-computer-generated curves showing penicillin G levels (as fraction of the IV dose) in the central and peripheral compartments of the two-compartment open model and the cumulative amount excreted in the urine as a function of time. The curves are based on the first-order rate constants associated with the model describing drug disposition kinetics in normal (solid line) and febrile (dashed line) dogs. From Baggot, 1980, with permission.

tributes more widely in febrile than in normal animals (Fig 4.8). Even though infectious diseases have in common the presence of fever, the alterations produced in drug disposition will vary with the pathophysiology of the disease. When corresponding changes occur in volume of distribution and clearance of a drug, the half-life remains unchanged (Abdullah and Baggot, 1984, 1986). Corresponding significant increases in both the volume of distribution and systemic clearance of trimethoprim administered in combination with sulfadimethoxine or sulfamethoxazole occurred in febrile pneumonic pigs compared with healthy pigs; the half-life of trimethoprim remained unchanged. The disposition kinetics of neither sulfonamide was

altered in the disease state (Mengelers et al., 1995). In the presence of an experimentally induced *E. coli* infection in pigs, the systemic clearance of enrofloxacin was significantly decreased while the volume of distribution remained unchanged. This resulted in an approximately 2.5-fold increase in the half-life of enrofloxacin (Zeng and Fung, 1997).

Changes in systemic clearance may occur when glomerular filtration is decreased (renal function impairment) or hepatic microsomal metabolic activity is altered. Alteration of blood flow to the organ of elimination may affect the clearance of antimicrobials. Halothane anaesthesia, for example, significantly decreased the clearance of gentamicin resulting in signif-

icantly higher plasma concentrations at 8 h after IV administration of the drug (Smith et al., 1988).

Chloramphenicol, metronidazole and erythromycin inhibit hepatic microsomal enzymes, while rifampin and various lipid-soluble drugs (e.g., phenobarbital) and xenobiotics induce hepatic microsomal enzymes. Prolonged fasting (> 48 h), which is accompanied by hyperbilirubinemia, appears to decrease hepatic microsomal metabolic activity and thereby the rate of oxidative reactions and glucuronide synthesis. Although chronic liver disease or altered hepatic function can change the disposition of drugs that undergo extensive hepatic metabolism, indicator tests that would quantify the affected elimination process are not available for clinical application.

There is limited information regarding the influence of disease states, including gastrointestinal disease, on drug absorption. Decreased cardiac output (a feature of congestive heart failure) and, as a consequence, altered blood flow to the intestinal tract may influence the rate but probably not the extent of absorption (systemic availability). Combined IV and oral dose studies (i.e., determination of absolute bioavailability) are required to differentiate between altered absorption and disposition processes.

Dosage Regimen

Factors which affect drug dosage regimens are discussed in Chapter 5. A dosage regimen entails the administration of a series of maintenance doses at a constant dosage interval. Additional features relating to clinical efficacy are the dosage form, which determines the route of administration, of the drug selected and the duration of therapy. The dosing rate and duration of therapy should be appropriate to treat the infection. It is because bacterial susceptibility can be determined in vitro and values of the pharmacokinetic parameters describing bioavailability and disposition are known that dosing rates for antimicrobial agents can be calculated. The minimum effective plasma/serum concentration used in calculating the usual dosing rate for an antimicrobial is based on the MIC for the majority of pathogenic micro-organisms that are susceptible to the drug. Variation in the degree of infection and in drug concentrations attained at various sites of infection is partly catered for by the range of doses that is recommended for use in an animal. With the notable exception of penicillins, the maximum dose that can safely be administered does not generally exceed five times

the dose that would provide minimum effective plasma concentrations. It is usual to estimate the dose that would provide safe and effective plasma concentrations for an 8- or 12-hour dosage interval, depending on the (apparent) half-life of the antimicrobial agent.

Even though antimicrobial agents do not have a defined range of clinically effective plasma concentrations, a dosing rate based on maintaining a desired average steady-state plasma concentration throughout the dosage interval is a useful approach to therapy, especially for drugs that produce a bacteriostatic effect. They include tetracyclines, macrolides and lincosamides, sulfonamides when used alone, chloramphenicol and its derivatives. The dosing rate of a drug can be defined as the systemically available dose ($F \times \text{Dose}$) divided by the dosage interval (τ):

$$\begin{aligned}\text{Dosing rate} &= \frac{F \times \text{Dose}}{\text{Dosage interval}} \\ &= C_{p(\text{avg})} \times \text{CL}_B\end{aligned}$$

where $C_{p(\text{avg})}$ is the average plasma concentration of the drug at steady-state associated with multiple dosing at a fixed (selected) dosage interval and CL_B is the systemic clearance of the drug. The relationship can be applied only to drugs that show linear pharmacokinetic behavior, that is, absorption and elimination are first-order processes. The desired average steady-state plasma concentration is a multiple of the MIC_{90} ; the multiple represents the area under the inhibitory curve (AUC) associated with the dosage interval. This approach may be applied to the calculation of dosing rates for fluoroquinolones, which produce a concentration-dependent effect on Gram-negative aerobic bacteria, using a number within the range 3 to 5 as the multiple of MIC_{90} . Because only the free (unbound to plasma proteins) drug is microbiologically active, the plasma drug concentration (measured as total drug) may be corrected for the extent of protein binding by calculating the fraction unbound (f_u) and expressed as free drug concentration in the plasma. This refinement is clinically worthwhile but infrequently applied. Assuming knowledge of the systemic availability and the clearance of a drug, the average plasma concentration at steady-state that would be achieved by applying a fixed dosing rate can be predicted:

$$C_{p(\text{avg})} = \frac{F \times \text{Dose}}{\text{CL}_B \times \tau}$$

The longer the dosage interval (τ) relative to the half-life of the drug, the greater will be the degree of fluctuation in plasma concentrations at steady-state. By selecting a dosage interval similar to the (apparent) half-life of the drug, fluctuation in plasma concentrations will be minimized; fluctuation would be non-existent when the drug is administered by IV infusion.

When a drug is administered by IV infusion, the clearance of the drug determines the rate of infusion (R_o) that would be required to produce a desired steady-state (plateau) concentration:

$$R_o = C_{ss} \times Cl_B$$

The steady-state plasma concentration achieved by continuous infusion or the average plasma concentration at steady-state produced by multiple dosing at a constant dosage interval depends on the clearance of the drug. The time required to attain steady-state depends solely upon the half-life of the drug. After infusing the drug solution for a period corresponding to 4 times the half-life, the plasma concentration will be within 90 percent of the eventual steady-state concentration. Some third generation cephalosporins (e.g., cefotaxime, ceftazidime) and ampicillin are among the relatively few antimicrobial agents for which continuous intravenous infusion is a feasible method of administration to animals. Steady-state can be attained either gradually by continuous infusion or multiple dosing or promptly by administering a loading dose. The size of the loading dose that would provide a desired plasma concentration can be calculated:

$$\text{Loading dose} = C_{p(\text{avg})} \times V_d(\text{area})$$

Alternatively, the loading dose can be based on the fraction of drug eliminated during the dosage interval and be related to the maintenance dose of the dosage regimen. This approach is generally applied to antimicrobial agents that produce a bacteriostatic effect and have half-lives between 8 and 24 hours (e.g., conventional dosage forms of sulfonamides and tetracyclines).

Duration of Therapy

The success of antimicrobial therapy depends upon the administration of multiple doses, at an appropriate dosage interval, of a drug to which the causative pathogenic micro-organisms are susceptible at the concentrations attained at the site of the infection and

also on the duration of treatment. While both the microbiological and pharmacokinetic properties of the antimicrobial agent selected are taken into account in the dosage regimen, the duration of treatment is largely empirical. It is imperative that antimicrobial therapy be maintained for an adequate duration, which should be based upon monitoring the response both by clinical assessment of the animal patient (resolution of fever, leukocytosis and other signs of acute inflammation) and bacterial culture of properly collected specimens. Definitive diagnosis at an early stage of infection and the application of specific therapy, based on knowledge of the causative pathogenic micro-organism and its susceptibility, will decrease the overall duration of treatment and minimize residual sequelae. An extended course of treatment is generally required in immunocompromised animals. Because of their potential to produce toxicity, due to preferential accumulation associated with selective binding to phospholipid (phosphatidylinositol)-rich tissues of the inner ear and kidney cortex, and ability (with the possible exception of amikacin) to induce plasmid-mediated bacterial resistance, therapy with an aminoglycoside antibiotic should not be extended beyond the duration required to treat the infection.

There are certain infections which, due to the relative inaccessibility of the causative pathogenic micro-organisms to antimicrobial agents, invariably require a prolonged duration (3 to 5 weeks, rather than 6–10 days) of therapy. They include prostatitis, osteomyelitis and skin infections in dogs, and *Rhodococcus equi* pneumonia in foals. In the treatment of these infections and of urinary tract infections, which requires at least 2 weeks of therapy, preference should be given to the use of orally effective antimicrobial agents.

Development of Antimicrobial Preparations

Blood concentration profiles generated at the low and high ends of the approved dose range, coupled with MIC data for commonly isolated bacterial pathogens, provide a basis for selection of the appropriate dose to use for the particular disease, organ system affected, and causative pathogenic micro-organism. The dose range may be defined by a clinically confirmed dose at the lower end and target species safety (including consideration of human food safety for food-producing animals) at the upper end of the dose range (Martinez and Berson, 1998).

Pharmacokinetic-pharmacodynamic (PK-PD) rela-

tionships have been well described for many antimicrobials and provide the basis for development of veterinary drug product labels that bear a range of doses. In terms of PK-PD relationship, antimicrobial agents may be ascribed to one of three general categories: (1) time-dependent bactericidal action, (2) concentration-dependent bactericidal action, and (3) bacteriostatic antimicrobial action (Craig, 1993). This topic is discussed extensively in Chapter 5.

Penetration into Cerebrospinal Fluid

The distribution of drugs from the blood into the central nervous system is unique because functional barriers, the blood-brain and blood-CSF barriers, are present that restrict entry of drugs into the CNS. Because brain capillary endothelial cells and choroidal epithelial cells have continuous tight junctions between adjacent cells, drug entry into the brain interstitial fluid and cerebrospinal fluid depends entirely on transcellular transport for which lipid solubility is a prerequisite. Drug penetration of the blood-CSF barrier is influenced by the concentration and rate of decline of the drug in blood plasma, the extent of binding to plasma proteins, and for drugs that are weak organic electrolytes, their degree of ionization in plasma (which is determined by pKa) and lipid solubility of the nonionized moiety. Lipid-soluble, nonionized drug molecules that are free (not bound to plasma proteins) in the blood plasma may enter brain interstitial and cerebrospinal fluids by passive diffusion.

Antimicrobial agents that penetrate the blood-CSF barrier include cefuroxime, cefotaxime, ceftazidime, ciprofloxacin, trimethoprim, sulfamethoxazole, sulfadiazine, metronidazole, chloramphenicol and fluconazole (triazole antifungal agent). In the presence of meningeal inflammation and fever, the penetrative capacity of these antimicrobial agents is increased and penicillin G, which poorly penetrates the uninflamed meninges, may attain concentrations in CSF adequate to treat infection caused by susceptible micro-organisms.

Drugs may leave the CSF by bulk flow into the venous sinuses, by passive diffusion of the nonionized (lipid-soluble) form into the blood and, in addition, there are efflux carriers present in the choroid plexus that actively secrete the ionized form of organic acids from CSF into the blood. When the meninges are inflamed, carrier-mediated active transport of penicillins from the CSF to the blood is impaired (Spector and Lorenzo, 1974).

Passage into Milk

The bovine udder is richly supplied with blood mainly through the external pudendal arteries and supplemented by a subsidiary supply, cranially through the subcutaneous abdominal artery and caudally via the perineal artery. The ratio of the volume of blood circulating through the mammary gland to volume of milk produced has been estimated to be 670:1, at a moderate level of milk production. This provides ample opportunity for the unbound fraction of lipid-soluble drugs to passively diffuse from the systemic circulation into milk. The passage of antimicrobial agents into milk shows the influence of chemical nature, degree of ionization and lipid solubility, and extent of plasma protein binding on the equilibrium concentration attained across a cellular barrier. The validity of using the milk-to-plasma equilibrium concentration ratio for predictive purposes is highly dependent on the experimental design applied in obtaining the results. Steady-state can be achieved either by infusing the drug intravenously for a period exceeding 4 times the half-life or by administering a loading dose followed by maintenance doses, each one-half the loading dose, at intervals equal to the half-life of the drug. After attaining equilibrium, blood and milk samples should be collected at regular (30-minute) intervals and drug concentration be determined in ultrafiltrates of plasma and milk.

The majority of antimicrobial agents cross the blood-milk barrier, which is a somewhat restrictive functional rather than an anatomical barrier, by passive diffusion. Both nonpolar lipid-soluble compounds and polar substances that possess sufficient lipid solubility passively diffuse through the predominantly lipoidal barrier. The rate of transfer is directly proportional to the concentration gradient across the barrier and the lipid solubility of the drug. The equilibrium concentration ratio of total (nonionized plus ionized) drug is determined by the degree of ionization in blood and milk, the charge on the ionized moiety, and the extent of binding to plasma proteins and milk macromolecules. It has been shown that only the lipid-soluble, nonionized moiety of a weak organic acid or base that is free (not bound to protein) in the plasma can penetrate cell membranes, enter the milk and diffuse into transcellular fluids. The milk-to-plasma equilibrium concentration ratio ($R_{m/p}$) can often be predicted (Rasmussen, 1966):

Table 4.9. Comparison of calculated and experimentally obtained milk:plasma concentration ratios for antimicrobial agents under equilibrium conditions.

Drug	Lipid Solubility	pK _a	Milk pH	Concentration Ratio (milk ultrafiltrate: plasma ultrafiltrate)	
				Theoretical	Experimental
Acids					
Penicillin G	Low	2.7	6.8	0.25	0.13–0.26
Cloxacillin	Low	2.7	6.8	0.25	0.25–0.30
Ampicillin	Low	2.7, 7.2	6.8		0.24–0.30
Cephaloridine	Low	3.4	6.8	0.25	0.24–0.28
Cephaloglycin	Low	4.9	6.8	0.25	0.33
Sulfadimethoxine	Moderate	6.0	6.6	0.20	0.23
Sulfadiazine	Moderate	6.4	6.6	0.23	0.21
Sulfamethazine	Moderate	7.4	6.6	0.58	0.59
Bases					
Tylosin	High	7.1	6.8	2.00	3.5
Lincomycin	High	7.6	6.8	2.83	3.1
Spiramycin	High	8.2	6.8	3.57	4.6
Erythromycin	Very high	8.8	6.8	3.87	8.7
Trimethoprim	High	7.3	6.8	2.32	2.9
Aminoglycosides	Low	7.8 ^a	6.8	3.13	0.5
Spectinomycin	Low	8.8	6.8	3.87	0.6
Polymyxin B	Very low	10.0	6.8	3.97	0.3
Amphoteric					
Oxytetracycline	Moderate	—	6.5–6.8	—	0.75
Doxycycline	Moderate/high	—	6.5–6.8	—	1.53
Rifampin ^b	Moderate/high	7.9	6.8	0.82	0.90–1.28

^aThe pK_a value given for aminoglycosides is unconfirmed.^bTheoretical concentration ratio for rifampin is based on its behavior as an organic acid (pK_a 7.9).

For an acid,

$$R_{\text{milk/plasma}} = \frac{1 + 10^{(\text{pH}_m - \text{pK}_a)}}{1 + 10^{(\text{pH}_p - \text{pK}_a)}}$$

Or for a base,

$$R_{\text{milk/plasma}} = \frac{1 + 10^{(\text{pK}_a - \text{pH}_m)}}{1 + 10^{(\text{pK}_a - \text{pH}_p)}}$$

where pH_m and pH_p are the pH reactions of milk and plasma, respectively, and pK_a is the negative logarithm of the acidic dissociation constant of an organic acid or base. In normal lactating cows (milk pH range 6.5–6.8), weak organic acids attain milk ultrafiltrate-to-plasma ultrafiltrate concentration ratios less than or equal to 1; organic bases, excluding aminoglycosides and spectinomycin (which are polar), attain

equilibrium concentration ratios greater than 1 (Table 4.9). Some lipophilic bases concentrate (ion-trapping effect) in milk; these drugs have an advantage over other antimicrobial agents in the systemic treatment of mastitis.

The significance of this favored distribution decreases with increasing pH of milk, particularly for macrolides (Table 4.10). The higher pH of mastitic milk (6.9–7.2) does not interfere with antibacterial activity of macrolides and aminoglycosides, whereas their activity would be decreased in a more acidic environment. An undesirable feature of the distribution of macrolides is diffusion from the systemic circulation into ruminal fluid (pH 5.5–6.5), where the ion-trapping effect also applies. Because spiramycin avidly binds to tissue components, the persistence of drug residues is a major disadvantage associated with its use.

Table 4.10. Comparison of the fraction of dose recovered in normal and mastitic milk for antibiotics administered intramuscularly to cows.

i		Percentage Non-ionized in Plasma	Percentage of Dose Recovered in Milk	
Drug	pK _a		Normal	Mastitic
Acids				
Penicillin G	2.7	0.002	0.001	0.001
Cloxacillin	2.7	0.002	0.001	0.001
Ampicillin	2.7, 7.2		0.08	0.10
Amoxicillin	2.7, 7.2		0.06	0.15
Bases				
Tylosin	7.2	66.67	2.60	1.40
Spiramycin	8.2	13.68	6.80	2.40
Erythromycin	8.8	3.85	3.80	2.20
Spectinomycin ^a	8.8	3.85	0.04	0.08
Gentamicin ^a	7.8	28.47	0.006	0.01
Polymyxin	10.0	0.25	0.001	0.001
Amphoteric				
Oxytetracycline	—	—	0.07	0.08
Doxycycline	—	—	0.15	0.15

^aPolar drug with low solubility in lipid.

Lipid solubility appears to be the principal factor that governs the passage of tetracyclines (amphoteric compounds) into milk and the equilibrium concentration ratios attained. Even though doxycycline is 85–90% bound to plasma proteins and oxytetracycline is only 20% bound, the equilibrium concentration ratio of doxycycline is 1.53 while that of oxytetracycline is 0.75 at milk pH within the range 6.5–6.8. Tetracyclines exert their greatest activity at an acidic pH close to their isoelectric point (5.5 for all tetracyclines apart from minocycline, 6.0). This implies that their antimicrobial activity would be less in mastitic milk (pH 6.9–7.2).

Enrofloxacin and its active metabolite ciprofloxacin, formed by N-deethylation (a microsomal-mediated oxidative reaction) in the liver, would be expected to attain concentrations in milk that would be effective against Gram-negative aerobic bacteria, in particular *Escherichia coli* (Kaartinen et al., 1995). Ceftiofur, 3 mg/kg administered by intramuscular injection, at 12-hour dosage intervals, could be used for treatment of acute coliform mastitis. This antimicrobial agent has time-dependent bactericidal activity against the causative bacterial pathogens and is resistant to beta-lactamases that may be produced by these bacteria.

The response to systemic treatment with ceftiofur would mainly depend on the concentration of the drug and its active metabolite (desfuroylceftiofur) attained in the mammary gland. The concentration that would be attained in milk is influenced by the dosing rate and limited both by relatively low barrier membrane penetrative capacity and extensive binding to plasma proteins.

Flunixin meglumine (2.2 mg/kg), administered by intravenous injection at 24-hour dosage intervals, may have a place in the treatment of acute *E. coli* mastitis when the infection is diagnosed at an early stage. The nonsteroidal anti-inflammatory drug does not interfere with the activity of concomitantly used antimicrobial agents. Frequent stripping of the infected quarter(s) to remove bacteria and cellular debris is important, perhaps essential, in coliform mastitis. The slow intravenous injection of oxytocin, 5 to 10 units of diluted solution (10 units/ml), facilitates the completeness of stripping (milkout).

The principal differences in mammary gland physiology are in the relative volume of milk produced by various species and in the composition of the milk, particularly the fat (triglycerides) and protein (casein) content.

Considerations in Pregnant Animals

Physiological adaptations that occur during pregnancy and could influence the oral bioavailability and disposition of drugs include an increase in gastric pH, an increase in the circulating blood (plasma) volume and in renal blood flow, an alteration in body fluid compartments, and hormone-induced change in hepatic microsomal enzyme activity. A major concern in the use of drugs during pregnancy is the potential for adverse effects on the fetus, since all drugs administered to the mother cross the placental barrier, although at different rates, and the fetus is ill-equipped to eliminate drugs. To what extent enzymes located in the placental membranes (e.g., microsomal drug-metabolizing system, which mediates various oxidative reactions, and cholinesterase) contribute to the conversion of drugs to inactive, more active or potentially toxic metabolites does not appear to have been established in domestic animal species.

Placental drug transfer by passive diffusion is similar to passage across any epithelial barrier, and in many respects resembles passage from the systemic circulation into milk of lactating animals. Since the pH of ar-

Table 4.11. Suggested cautions or contraindications of potentially toxic antimicrobial drugs in pregnant animals.

Drug	Toxicity	Recommended Use	
		Caution*	Contraindicated
Antibacterial			
Aminoglycosides	Auditory nerve toxicity?	+	
Chloramphenicol	Gray syndrome in newborn.	+	
Fluoroquinolones	Arthropathy in immature animals.	+	+
Metronidazole	Carcinogenic in rodents?	+	At term
Nitrofurantoin	Hemolytic anemia in newborn.	+	
Sulfonamides	Increased risk neonatal jaundice, teratogenic in some studies.	+	
Tetracyclines	Tooth discoloration; inhibited bone growth in fetus; hepatic toxicity in pregnant animals with impaired renal function.		+
Trimethoprim	Folate antagonist may cause congenital anomalies.	+	
Antifungal			
Imidazoles, triazoles	Teratogenic.	+	
Griseofulvin	Teratogenic.		+

*Caution: Do not use if suitable alternative is available.

terial blood in the fetus (7.27) is only slightly lower than in the mother (7.37), the ion-trapping effect whereby lipophilic organic bases attain higher concentrations in the milk does not apply to the fetal circulation. Drug diffusion across the placenta from mother to fetus is favored by lipid solubility, a large concentration gradient of unbound drug between the maternal and fetal circulations, and the presence of drug in the nonionized form in the maternal circulation. Blood flow to the placenta limits the rate of delivery of drug to the fetal circulation. Conversely, molecules that are ionized (penicillins, cephalosporins), hydrophilic (aminoglycosides), and present in low free drug concentrations (doxycycline, macrolides, lincosamides) have restricted access to the fetus. Differences in the extent of plasma protein binding by the mother and fetus (in which it is lower) affect the total plasma drug concentrations in the maternal and fetal circulations. Regardless of the physicochemical properties of a drug, the duration of maternal therapy with the drug influences the concentrations that will be attained in the fetus.

Because toxic effects could be produced in the fetus, caution should be exercised with the use in pregnant animals of a wide variety of antimicrobial agents (Table 4.11), while some others (fluoroquinolones, tetracyclines, griseofulvin) are contraindicated.

When selecting an antimicrobial for administration to a pregnant animal, due consideration must be given

to the potential of some of these drugs to produce adverse effects on the fetus.

Renal Excretion

Polar drugs and drug metabolites have restricted extravascular distribution, which may be largely confined to extracellular fluid, and undergo elimination by renal excretion. This is because of their limited capacity to passively diffuse through lipid membranes. Even though lipid-soluble drugs are mainly eliminated by hepatic metabolism, a fraction of the systemically available dose is usually eliminated by renal excretion. Because herbivorous species metabolize most lipid-soluble drugs more rapidly than carnivorous species, a smaller fraction of the dose is eliminated by renal excretion in herbivorous species, for example, trimethoprim (Table 4.2).

The renal excretion of drugs and drug metabolites involves glomerular filtration and, for some drugs and most metabolites, carrier-mediated proximal tubular secretion. Extensive binding to plasma proteins limits the availability of drug molecules for glomerular filtration but might not hinder their secretion by proximal renal tubules because of rapid dissociation of the drug-protein complex. The glomerular filtration rate (GFR) varies among animal species. Average values of GFR, expressed in units of mL/min \times kg body weight are: horse, 1.65; sheep, 2.20; cattle and goats, 2.25; pig, 2.80; cat, 2.94; dog, 3.96. At least in companion animal

species (horses, dogs and cats), endogenous creatinine clearance provides a clinically useful index of renal function (GFR).

Aminoglycoside antibiotics are eliminated almost entirely by glomerular filtration. Their half-lives reflect the relative rates of glomerular filtration in domestic animal species; the higher the glomerular filtration rate, the shorter the half-life of an aminoglycoside. The half-life of gentamicin, for example, is 1.25 h in dogs, 1.8 h in cattle, and 2.6 h in horses. The primary route of elimination for most tetracyclines including oxytetracycline is renal excretion. Doxycycline and minocycline, which are more lipid-soluble than other drugs in this class, are exceptions in that doxycycline is excreted in the feces as an inactive conjugate or chelate and minocycline may be eliminated mainly by metabolism. Enterohepatic circulation largely accounts for the relatively slow elimination of oxytetracycline which takes place by glomerular filtration. Fluconazole, unlike other azole antifungal drugs, is eliminated by renal excretion.

The majority of beta-lactam antibiotics (penicillins and cephalosporins) are eliminated both by glomerular filtration and proximal tubular secretion. Nafcillin (a staphylococcal penicillinase-resistant penicillin), ceftriaxone and cefoperazone (third-generation cephalosporins) are exceptions in that they are mainly excreted by the liver in bile. Ceftiofur, following absorption into the systemic circulation, is converted by ester hydrolysis to desfuroylceftiofur which has antibacterial activity similar to that of the parent drug.

Ciprofloxacin, a drug in its own right and the active metabolite of enrofloxacin, is mainly eliminated by renal excretion (glomerular filtration and proximal tubular secretion). Probenecid, by inhibiting renal tubular secretion, decreases the renal clearance of penicillins and ciprofloxacin. The beta-lactamase inhibitor clavulanic acid does not alter the disposition (i.e. distribution and elimination) of the penicillins (amoxicillin and ticarcillin) with which it is combined.

While a drug may enter tubular fluid both by glomerular filtration and proximal tubular secretion, its renal clearance may also be influenced by reabsorption from the distal nephron. As tubular reabsorption takes place by passive diffusion, it is influenced by lipid solubility and concentration of the drug in distal tubular fluid, and by the pKa/pH determined degree of ionization of weak organic acids and bases. The reabsorption of weak organic acids and bases is confined to the

lipid-soluble non-ionized form of these drugs. Alkalinization of the urine, by favoring ionization of weak organic acids (e.g., sulfamethoxazole, pKa 6.0; sulfadiazine, pKa 6.4) in distal tubular fluid, may increase their elimination while it may decrease the elimination, by promoting reabsorption, of weak organic bases (e.g., trimethoprim, pKa 7.3). At urine pH reactions of 6.0 and 8.0, the percentages of sulfadiazine that exist in the lipid-soluble nonionized form are 71 and 2.4, respectively, and of trimethoprim are 5 and 83.4, respectively. The urine pH-excretion rate dependency is significant only when the fraction of the dose excreted in urine exceeds about 20% and the nonionized moiety in distal tubular fluid is lipid-soluble.

Renal Impairment

Renal disease decreases the rate of elimination of drugs that are cleared predominantly by the kidneys. Reduced glomerular filtration rate decreases the elimination of penicillins (except nafcillin), cephalosporins (except ceftriaxone and cefoperazone), ciprofloxacin, fluconazole and especially aminoglycosides. While reduced GFR decreases the elimination of tetracyclines, with the notable exception of doxycycline, changes in extravascular (tissue) distribution may exert an influence. Doxycycline, unlike other tetracyclines, is entirely eliminated by non-renal mechanisms and does not accumulate significantly in the presence of renal failure. These features make doxycycline the tetracycline of choice for use in dogs (half-life 7.0 hours) and cats (half-life 4.6 hours) with renal function impairment without a need to adjust the dosing rate. Renal blood flow can influence all of the processes involved in the excretion of drugs, but changes in renal blood flow are likely to have a more pronounced effect on tubular processes than on glomerular filtration. Because of its narrow therapeutic index and high potential to cause nephrotoxicity associated with a dose-related decrease in renal blood flow, dosage with amphotericin B requires particular attention, and renal function must be monitored during the course of treatment of systemic mycoses with this drug.

In the presence of impaired renal function, modification of the usual dosing rate of an aminoglycoside antibiotic may be required to prevent accumulation of the drug with an increased risk of producing either ototoxicity or nephrotoxicity, or both. The dosing rate of an aminoglycoside should be adjusted in accor-

dance with the decrease in renal function. An indication of the magnitude of the decrease in GFR may be obtained by measuring endogenous creatinine clearance in the animal. Dosage adjustment may be made either by reducing the dose and maintaining the usual dosage interval or by administering the usual dose at a longer dosage interval; the latter adjustment is preferable. Whatever dosing rate is used, trough plasma concentrations of gentamicin should not be allowed to exceed 2 $\mu\text{g/mL}$. Because dehydration enhances the toxicity of aminoglycosides, the concurrent use of an aminoglycoside and a diuretic agent, especially furosemide which also has ototoxic potential, should be avoided. The nephrotoxic potential of aminoglycosides is influenced both by the dosing rate and the duration of therapy, which should not be extended beyond that required to cure the infection.

The dosage interval for fluconazole should be increased in the presence of impaired renal function. The adjustment could be based on the decrease in creatinine clearance. Depending upon the degree of renal impairment, consideration should be given to dosage adjustment of enrofloxacin that would allow for the decreased rate of excretion of ciprofloxacin. Since a significant fraction of the systemically available dose of marbofloxacin is eliminated by renal excretion, adjustment of dosage should be considered in the presence of renal impairment. Even though beta-lactam antibiotics, especially penicillins, have a wide margin of safety, the size of the dose should be decreased, depending on the decrease in creatinine clearance, in animals with renal failure. Since nafcillin, ceftriaxone and cefoperazone are excreted by the liver in bile, dosage adjustment is not required in renal insufficiency.

In the presence of uremia associated with chronic renal failure, the binding of acidic drugs to plasma albumin is reduced and the rate of certain biotransformation pathways (e.g., reductive and hydrolytic reactions) is decreased. The significance of these alterations on the activity and dosage of antimicrobial agents that could be affected remains to be determined. It is likely that the activation of prodrugs (such as pivampicillin) would be decreased. The hydrolytic conversion of cefotiofur to desfuoylcefotiofur could be decreased.

Modification of Dosage Regimens

The primary pathophysiologic sequelae relevant to antimicrobial dosing in renal dysfunction is a decreased glomerular filtration rate (GFR), which results in a de-

creased clearance of drugs eliminated by the kidney. Because of the large renal functional reserve, 75% of GFR must generally be lost before signs of clinical disease are readily evident. Adjustments to dosage regimens generally account only for decreased GFR, and unless therapeutic drug monitoring (TDM) is employed, other changes seen in severe renal dysfunction will not be accounted for.

The construction of modified dosage regimens in renal failure assumes that renal drug clearance directly correlates with clinical estimates of GFR (e.g., creatinine clearance or $1/\text{serum creatinine}$), that the intact nephron hypothesis holds true, and that relative glomerular-tubular balance is present. In these cases, an antimicrobial's renal clearance is a linear function of GFR independent of whether the drug is filtered, secreted, and/or absorbed in the kidney. In addition, the volume of distribution of the drug is assumed to be unchanged.

When TDM is available, both a drug's clearance and volume of distribution may be directly determined in an individual pharmacokinetic study. The resulting individualized dosing regimen thus accounts for the renal insufficiency present. However, even in this scenario, as is true for other approaches, the shape of the serum concentration-time profile in an animal with renal failure cannot be made to precisely duplicate that in a healthy animal since the drug's clearance is reduced and half-life prolonged (Frazier and Riviere 1987). In general, TDM is only employed for toxic antimicrobials whose accumulation would adversely affect the animal's health. A great deal of effort, both in veterinary and human medicine, has therefore been spent on the nephrotoxic aminoglycoside antibiotics. The effort is further necessitated by the great variability often seen in aminoglycoside pharmacokinetics in diseased animals where both creatinine clearance (Cl_{Cr}) and fluid status (V_d) are often changed (Frazier et al., 1988), necessitating close monitoring to avoid drug-induced nephrotoxicity.

The initial loading dose of the drug should be the same as in the normal animal. Dose-reduction schemes attempt to decrease the subsequent maintenance doses or increase the dosing interval, both in proportion to decreased Cl_{Cr} . Table 4.12 lists the antimicrobial agents commonly used in veterinary medicine for which modified dosage regimens can be formulated on the basis of existing data. Extrapolation from human studies is often necessary because of a lack of such work in

Table 4.12. Antimicrobial drug dosage adjustments for renal failure.

Drug class	Examples	Route of elimination	Dosage adjustment
Aminoglycosides	Amikacin, gentamicin, tobramycin	Renal	Interval extension. ^a
Cephalosporins	Cefazolin, cephalexin,	Renal	Interval extension.
	Cefaclor	Hepatic	No change.
Lincosamides, macrolides	Cephalothin	Renal, hepatic	2x interval with severe renal failure.
	Clindamycin	Hepatic	No change.
	Erythromycin, tylosin	Hepatic, renal	No change.
	Lincomycin	Hepatic, renal	3x interval in severe renal failure.
Fluoroquinolones	Ciprofloxacin, enrofloxacin	Renal	Dosage reduction.
Penicillins	Cloxacillin, oxacillin	Hepatic	No change.
	Ampicillin, amoxicillin, carbenicillin, penicillin G,	Renal, hepatic	Half dose or 2x interval in severe renal
	ticarcillin, clavulanic acid, imipenem-cilastatin		failure.
Phenicol	Chloramphenicol	Hepatic	No change, but avoid in renal failure.
Polymyxins	Polymyxin B		Contraindicated.
Sulfonamides	Sulfisoxazole	Renal, hepatic	2-3x interval in severe renal failure.
	Trimethoprim-sulfamethoxazole	Renal, hepatic	No change, but do not use in severe renal failure.
Tetracyclines	Tetracyclines	Renal, hepatic	Contraindicated, except for doxycycline.
	Doxycycline	GI mucosa	No change.
Miscellaneous	Amphotericin	Hepatic	Half dose in severe renal failure.
	Metronidazole	Hepatic, renal	Unknown.

^aIndividual therapeutic drug monitoring should be used when available because of variability in disposition.

animals. For drugs eliminated primarily by hepatic mechanisms (e.g., chloramphenicol) or drugs with wide safety indices (e.g., penicillins), dosage modification is often unnecessary. In cases in which such antimicrobials would be efficacious, the drugs that are cleared by hepatic mechanisms are preferred. When modification based on Cl_{Cr} is indicated, the following two methods are suggested.

- (1) Interval extension: Administer normal maintenance dose.

$$\text{Interval} = \text{Normal Interval} \times \frac{(\text{Normal } Cl_{Cr})}{(\text{Patient } Cl_{Cr})}$$

- (2) Dose reduction: Administer at the normal dose interval.

$$\text{Dose} = \text{Normal dose} \times \frac{(\text{Patient } Cl_{Cr})}{(\text{Normal } Cl_{Cr})}$$

In severe renal failure, use of the interval extension method may result in excessively prolonged periods of sub-inhibitory drug concentrations. In this case, half or one-third of the dose should be given at half or one-third, respectively, of the calculated intervals.

If Cl_{Cr} is not available, some researchers have suggested that $1/SCR$ (serum creatinine) or $1/BUN$ (blood urea nitrogen) be substituted. In severe renal failure, this may not be accurate.

Considerable effort has been expended to define how a dosage regimen in a diseased individual can be constructed to maintain efficacy and avoid toxicity. This may be an impossible goal, suggesting that trade-offs must be made. Close clinical monitoring is required to ensure antimicrobial efficacy and no drug-induced toxicity. The latter is especially difficult when detecting aminoglycoside-induced nephrotoxicity is confounded by the underlying renal dysfunction. For aminoglycosides, interval extension has been shown to better reduce toxicity than dose reduction. The data are not as clear for other drugs. Serial monitoring of renal function tests (SCR, BUN), urinary enzymes, or TDM are the only approaches that may be used.

Finally, it must be stressed that if renal disease is present in food-producing animals, the decreased GFR would be expected to result in a prolonged elimination half-life, possibly necessitating a prolonged withdrawal time. Guidelines have not been established in veterinary medicine to address this problem, other than using drugs not eliminated by the kidney or using

drugs with very short half-lives so that prolonged withdrawal times would suffice. Again, aminoglycosides, characterized by prolonged tissue half-lives, would be contraindicated. On-site urine monitoring could also be employed to reduce the chance of residues.

Urinary Drug Concentration

The concentration of a drug in the urine depends on the dose administered, the dosage form and route of administration, the extent of absorption (systemic availability) of the drug, the fraction of the systemically available drug excreted unchanged (as parent drug and/or active metabolite) in the urine, and the volume of urine produced which is related to the hydration status of the animal. The urine pH reaction (usual range is pH 5.5 to 7.0 in dogs and cats; pH 7.2 to 8.4 in horses) may influence antimicrobial activity. Fluroquinolones, with the probable exception of difloxacin, are more active against Enterobacteriaceae and other Gram-negative aerobic bacteria in an alkaline environment.

The success of treatment of urinary tract infections depends on the maintenance for most of the dosage interval of high urinary concentrations (at least four-fold the MIC) of an antimicrobial agent to which the causative pathogenic micro-organisms are at least moderately susceptible. Because a two-week course of treatment should be applied, a drug that can be administered orally at 12-hour intervals should be selected since poor owner compliance is a common cause of treatment failure. Amoxicillin-clavulanate, trimethoprim-sulfadoxine (or sulfadiazine) combination, cephalexin and enrofloxacin meet the requirements. Selection of the drug to use should be based on bacterial culture of a urine specimen collected before initiating treatment. The effectiveness of therapy can be assessed by bacterial culture of urine specimens collected between the fourth and sixth days after commencing treatment and between four and six days after completing the two-week course of treatment.

Elimination by the Liver

Most lipid-soluble drugs are eliminated by hepatic metabolism, principally by hepatic microsomal oxidation and glucuronide formation, and some are excreted unchanged (as parent drug) in bile. Antimicrobial agents that are mainly eliminated by hepatic metabolism include some fluoroquinolones (enroflox-

acin, difloxacin, marbofloxacin), trimethoprim, sulfonamides, minocycline, chloramphenicol and its derivatives, clindamycin, metronidazole, rifampin, and azole antifungal drugs with the notable exception of fluconazole. Macrolides and lincosamides, nafcillin, cefoperazone and ceftriaxone are eliminated by biliary excretion, while marbofloxacin is partly eliminated by excretion in bile as well as in urine. Even though tetracyclines (except minocycline and doxycycline) are excreted in bile they are reabsorbed from the intestine and returned to the liver (enterohepatic cycle) for re-entry into the systemic circulation.

Liver blood flow and capacity of the liver to eliminate lipid-soluble drugs by metabolism or excretion in bile are the principal factors that determine hepatic clearance. Extensive binding to plasma proteins may limit access of a drug to metabolizing enzymes within hepatocytes. Differences in the rates of hepatic microsomal oxidative reactions or in conjugate (especially glucuronide) formation often account for species variations in the hepatic clearance of a lipid-soluble drug. The extent to which differences in clearance affect half-life is influenced by the apparent volume of distribution of the drug, since half-life is a hybrid pharmacokinetic parameter.

Moderate or severe liver damage reduces the capacity of the liver to eliminate antipyrine (a marker substance for microsomal oxidative activity) and indocyanine green (marker substance for biliary secretion which may be influenced by liver blood flow). The uncertainty associated with quantification of the degree of hepatic dysfunction and its influence on the clearance of lipid-soluble drugs makes it difficult to predict dosage adjustment that might be required. In general, the dosage interval for a drug that is mainly eliminated by hepatic metabolism should be increased in the presence of impaired liver function, and preference should be given to the use of a drug that has a bactericidal action. Likewise, the dosage interval of an antimicrobial agent that is extensively metabolized by the liver should be increased when it is used concomitantly with a drug that inhibits microsomal oxidative reactions (such as ketoconazole, omeprazole or cimetidine).

A somewhat contrasting situation applies when griseofulvin and phenobarbital are used concomitantly in epileptic dogs in that the dose level (mg/kg) of phenobarbital may have to be increased in order to prevent convulsive seizures from occurring. Both

griseofulvin and phenobarbital induce hepatic microsomal oxidative activity. The rate of oxidative metabolism of metronidazole is increased by phenobarbital and rifampin.

Absorption and Disposition in Neonatal Animals

The neonatal period, which is generally considered to be the first month of postnatal life, varies among species. It appears to be 1 to 2 weeks in foals, about 8 weeks in calves, kids, lambs, and piglets, and 10 to 12 weeks in puppies. However, the most profound adaptive changes in physiological variables occur during the first 24 hours after birth in all species. This coincides with the time that the pharmacokinetic behavior of drugs is most "unusual" (Baggot and Short 1984). Some characteristics of the neonatal period include better absorption from the gastrointestinal tract, lower binding to plasma proteins, lower ratio of body fat-to-fluids, increased volume of distribution of drugs that distribute in extracellular fluid or total body water, increased permeability of the "blood-brain" barrier, and slower elimination (longer half-life) of most drugs.

Antimicrobial agents, such as penicillins, that are poorly absorbed and cause digestive disturbances in older foals (over 4 months of age) and adult horses can be administered orally to neonatal and young (up to 4 months of age) foals for the treatment of systemic bacterial infections caused by susceptible microorganisms. Oral administration of amoxicillin trihydrate (30 mg/kg), as a 5% oral suspension, to 5- to 10-day-old foals produced serum amoxicillin concentrations above 1 µg/ml for 6 hours (Love et al., 1981). Systemic availability of amoxicillin was 30 to 50% in the foals compared with 5 to 15% in adult horses (Baggot et al., 1988b). Pivampicillin, a prodrug of ampicillin, has systemic availability (ampicillin) of 40 to 53% in foals between 11 days and 4 months of age (Ensink et al., 1994). In adult horses, the systemic availability of ampicillin administered as pivampicillin is within the range 31 to 36%. The half-life of aminobenzyl penicillins is approximately twofold longer following oral than intravenous administration. It may be because of their moderate extent of absorption that the detrimental effect of oral penicillins, which is due to severe disturbance of the balance between the commensal bacterial flora in the colon of adult horses, is avoided in neonatal and young foals. There is no need to adjust the dosage interval in neonatal foals since penicillins in the systemic circulation have a wide margin of

safety. Penicillin V, the phenoxymethyl analog of penicillin G, does not have a place (due to low systemic availability and the production of digestive disturbances) in the treatment of bacterial infections in foals or adult horses (Baggot et al., 1990).

The systemic availability of cefadroxil (5% oral suspension) decreases progressively from 68% in 1-month-old foals to 14.5% in foals 5 months of age (Duffee et al., 1997). The half-life of the drug remains unchanged over this age range. Cephadrine, another first-generation oral cephalosporin, administered in sucrose syrup to 10- to 14-day-old foals has an average systemic availability of 64% and half-life of 1.1 h (Henry et al., 1992).

Since the rumen takes 4 to 8 weeks to develop and become functional, the bioavailability (rate and extent of absorption) of drugs administered orally to preruminant calves resembles that in monogastric species rather than in cattle. Although chloramphenicol is not approved for use in food-producing animals, a comparison between preruminant calves and neonatal foals is informative. Chloramphenicol, administered as an oral solution, is well absorbed in preruminant calves, and oral dosage (25 mg/kg at 12 h dosage intervals) will maintain therapeutically effective plasma concentrations (> 5 µg/ml) of the antibiotic (Huffman et al., 1981). In ruminant calves and adult cattle, orally administered chloramphenicol fails to provide effective plasma concentrations since the antibiotic is inactivated (reductive reaction) in the rumen. A single oral dose (50 mg/kg) of chloramphenicol solution administered to foals between 3 and 8 weeks of age produced an average peak plasma/serum concentration of 6 µg/ml, which was lower than the peak concentration produced in adult horses (18 µg/ml) given the drug at the same dose level (Buonpane et al., 1988). Changes in the disposition kinetics of chloramphenicol (administered as a single IV dose) are age-related, and the pattern of the change differs between species; a marked increase in the rate of chloramphenicol elimination (hepatic metabolism) during the first week after birth is a consistent finding (Table 4.13). Assuming that chloramphenicol is mainly eliminated by glucuronide conjugation, it would appear that this microsomal metabolic pathway develops far more rapidly in foals (within 1 week) (Adamson et al., 1991) than in calves (8 to 12 weeks) (Reiche et al., 1980). This finding is consistent with the shorter neonatal period in foals than in calves.

Table 4.13. Age-related changes in the disposition kinetics of chloramphenicol in calves (50 mg/kg IV) and foals (25 mg/kg IV).

Age	$V_{d(ss)}$ (mL/kg)	Cl_B (mL/min \times kg)	$t_{1/2}$ (h)
Calves (n=5)			
1 day	1130 \pm 50	1.1 \pm 0.24	11.7 \pm 1.7
7 days	1180 \pm 70	1.9 \pm 0.03	7.5 \pm 0.9
10 to 12 weeks	1230 \pm 60	3.1 \pm 0.63	4.9 \pm 0.7
Foals (n=6)			
1 day	992 \pm 269	2.25 \pm 0.67	6.19 \pm 2.43
3 days	543 \pm 173	6.24 \pm 2.22	1.48 \pm 0.51
7 days	310 \pm 67	8.86 \pm 1.90	0.64 \pm 0.14

Antimicrobial agents that undergo extensive first-pass metabolism by hepatic microsomal oxidative reactions would be expected to have higher systemic availability in neonatal animals. This applies to trimethoprim, which has far higher systemic availability in newborn kids than in older kids and adult goats. In addition to lower hepatic microsomal oxidative activity, the ruminal microflora have not developed in neonatal ruminant species.

Since disposition refers to the simultaneous effects of distribution and elimination, it is necessary to consider both components of the process when interpreting changes that occur during the neonatal period or in the presence of a disease state. Enrofloxacin is converted by N-dealkylation, a hepatic microsomal oxidative reaction, to ciprofloxacin. Both enrofloxacin and ciprofloxacin are active antimicrobially. Comparison of the disposition kinetics of enrofloxacin (2.5 mg/kg administered IV) in one-day-old and one-week-old calves shows that the volume of distribution at steady-state is smaller and the systemic clearance of the drug is lower in the one-day-old calves, while the half-life does not differ significantly between the one-day-old and one-week-old calves (Table 4.14). The changes in the disposition kinetics of enrofloxacin that occur during the first week of postnatal life in calves could be attributed to differences in plasma protein binding of enrofloxacin and in the body fat-to-fluids ratio since fluoroquinolones are lipid-soluble drugs. Newborn calves metabolize enrofloxacin to ciprofloxacin but the rate of formation of the active metabolite is slower and the peak serum concentration (C_{max}) is lower than in the one-week-old calves; mean t_{max} is about five times longer in newborn calves (Figure 4.9)

Table 4.14. Disposition kinetics of enrofloxacin and formation of ciprofloxacin in newborn and one-week-old Finnish Ayrshire calves.

Pharmacokinetic parameter	Age of calves		Statistical significance
	One day	One week	
Enrofloxacin			
$V_{d(ss)}$ (l/kg)	1.81 \pm 0.10 (1.54 – 2.01)	2.28 \pm 0.14 (1.88 – 2.52)	P = 0.035
Cl_B (l/h \times kg)	0.19 \pm 0.03 (0.14 – 0.28)	0.39 \pm 0.06 (0.31 – 0.56)	P = 0.021
$t_{1/2}$ (h)	6.61 \pm 1.12 (4.28 – 9.36)	4.87 \pm 0.68 (3.13 – 6.43)	Not significant
Ciprofloxacin			
t_{max} (h)	15.0 \pm 3.0 (12 – 24)	2.8 \pm 0.8 (1 – 4)	P = 0.007
C_{max} (mg/L)	0.087 \pm 0.017 (0.07 – 0.14)	0.142 \pm 0.005 (0.13 – 0.15)	P = 0.023

Note: A single dose (2.5 mg/kg) of enrofloxacin was administered by intravenous injection to the calves (n = 4 in each age group). Results are expressed as mean \pm SEM (and range).

(Kaartinen et al., 1997). Since the content of cytochrome P-450 has been shown to double during the first week of postnatal life in calves (Shoaf et al., 1987), it can be concluded that the rate of conversion of enrofloxacin to ciprofloxacin is age-related. Following IV administration of a single dose (2.5 mg/kg) of enrofloxacin, the sum of enrofloxacin and ciprofloxacin concentrations in plasma/serum was above 0.1 μ g/ml at 30 h and 24 h in one-day-old and one-week-old calves, respectively. The minimum inhibitory concentration for the majority of susceptible *E. coli* strains (MIC_{90}) isolated from calves is 0.25 μ g/ml.

Although there are species differences in the degree to which some drug metabolic pathways are deficient in neonatal animals, a relative lack of development of hepatic smooth-surfaced endoplasmic reticulum and its associated drug metabolizing enzyme systems (mediate oxidative reactions and glucuronide conjugation) appears to be a characteristic of the neonatal period in all mammalian species. Because of the low activity of most metabolic pathways, the half-lives of drugs that undergo extensive hepatic metabolism are prolonged in neonatal animals, particularly during the first 24 hours after birth. The maturation of the various metabolic pathways could be related to hormonal influence on postnatal enzyme induction. In the ma-

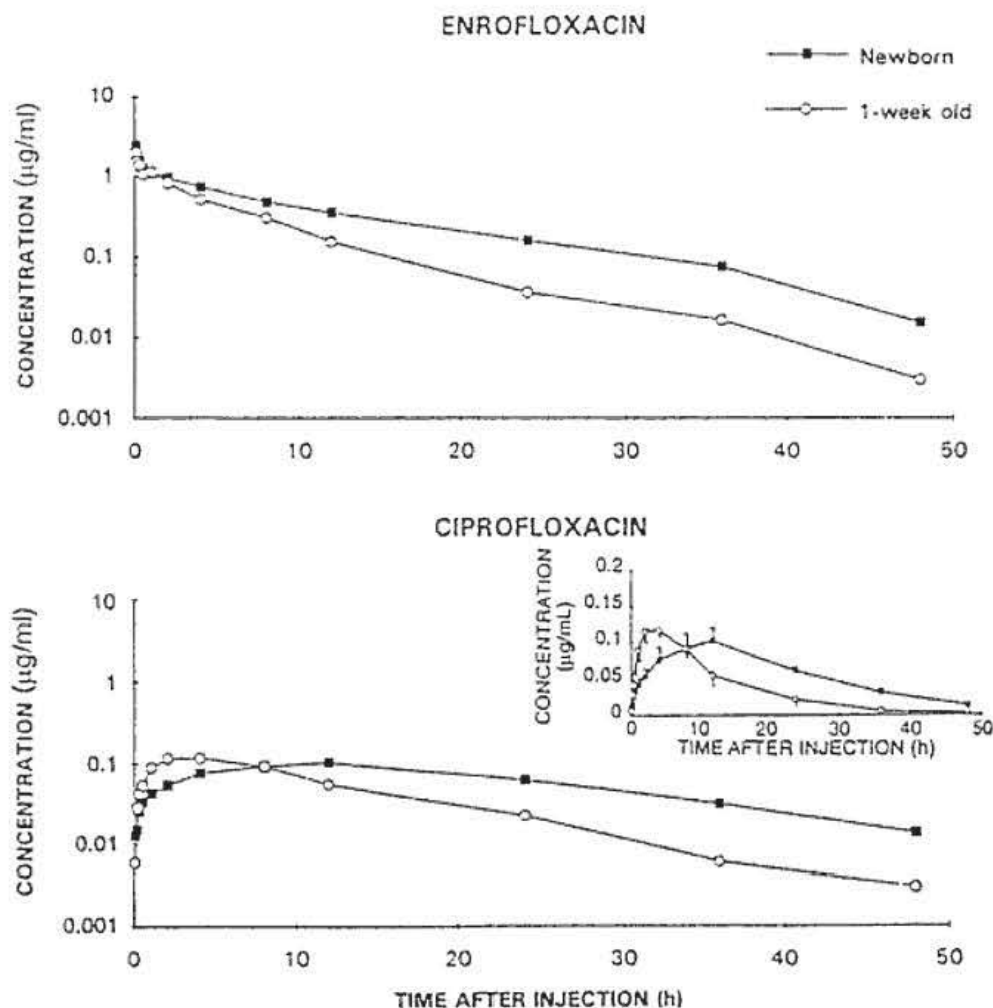


Figure 4.9. Mean concentration-time curves of enrofloxacin and its metabolite ciprofloxacin in newborn and one-week-old calves (4 calves per group). Enrofloxacin was administered IV at 2.5 mg/kg. Drug concentrations were analysed by HPLC. Lower panel insert: mean ciprofloxacin concentrations (\pm s.d.) on a non-logarithmic scale.

jority of species (ruminant animals, pigs, dogs, and presumably cats), the hepatic microsomal-associated metabolic pathways develop rapidly during the first 3 to 4 weeks after birth, and at 8 to 12 weeks of age have developed activity approaching that of adult animals (Nielsen and Rasmussen, 1976; Reiche, 1983). The foal appears to be an exception in at least the rate of development of glucuronide synthesis, which develops very rapidly during the first week after birth (Adamson et al., 1991). While a long dosage interval should be applied during the first 3 days after birth, it can gradually be decreased, depending on the animal species, as the neonate matures.

Conversion of ceftiofur to desfuroylceftiofur, a third-generation cephalosporin, is catalyzed by an es-

terase, which is most active in the kidneys followed by the liver (Olson et al., 1998). Desfuroylceftiofur has antibacterial activity similar to that of the parent drug, and the active metabolite rapidly becomes reversibly bound to proteins in plasma and tissues and forms conjugates with glutathione and cysteine. The high performance liquid chromatographic (HPLC) assay method measures the combined plasma concentration of ceftiofur and desfuroylceftiofur conjugates as a single derivative, desfuroylceftiofur acetamide, which is expressed as micrograms of ceftiofur free acid equivalents per ml (Jaglan et al., 1990). In a study of the influence of age on the disposition kinetics of ceftiofur, administered IV as ceftiofur sodium at a dose of 2.2 mg ceftiofur free acid equivalents per kg body weight,

Table 4.15. Pharmacokinetic values for plasma concentrations of ceftiofur and metabolites after IV injection of ceftiofur sodium in Holstein bull calves of various ages.

Age	$V_{d(ss)}$ (ml/kg)	Cl_B (mL/h \times kg)	$t_{1/2}$ (h)
1 week	345 \pm 62	17.8 \pm 3.2	16.1 \pm 1.5
1 month	335 \pm 92	16.7 \pm 3.1	17.2 \pm 3.1
3 months	284 \pm 49	30.3 \pm 4.6	8.2 \pm 2.8
6 months	258 \pm 72	39.8 \pm 14.9	5.95 \pm 1.2

Note: Dosage of ceftiofur sodium was 2.2 ceftiofur free acid equivalents per kilogram. Plasma concentrations of ceftiofur and metabolites were measured as desfuroylceftiofur acetamide by HPLC.

in Holstein bull calves, the volume of distribution at steady-state ($V_{d(ss)}$) decreased and systemic clearance (Cl_B) increased during the first 3 months after birth (Brown et al., 1996). The progressive decrease in volume of distribution of ceftiofur and desfuroylceftiofur conjugates could be attributed to the age-related decrease in extracellular fluid volume. The lower clearance in the 7-day-old and 1-month-old calves than in the older calves is probably due to maturation of the processes of elimination for ceftiofur and desfuroylceftiofur metabolites. Because the decreases in volume of distribution were proportionally less than the increases in clearance in calves 1 month of age and older, the half-life decreased more or less in accordance with the increased clearance (Table 4.15). Plasma concentrations of ceftiofur and its metabolites (measured as a single derivative) remained above the limit of quantification (LOQ, 0.15 μ g/ml) of the assay method for the entire 72 h blood sampling period in 7-day-old and 1-month-old calves, but decreased to below the LOQ within 48 h of drug administration to 6- and 9-month-old calves.

The renal excretion mechanisms (glomerular filtration and active, carrier-mediated tubular secretion) are incompletely developed at birth in all mammalian species. During the neonatal period renal excretion mechanisms mature independently at rates that are species-related. Glomerular filtration rate (GFR), based on inulin clearance, attains adult values at 2 days in calves, 2 to 4 days in lambs, kids and piglets, and may take at least 14 days in puppies. Proximal tubular secretion, based on clearance of para-aminohippurate, matures within 2 weeks after birth in the ruminant species and pigs, but may take up to 6 weeks in dogs. Indirect evidence, provided by pharmacokinetic stud-

ies of some antimicrobial agents, suggests that renal function develops rapidly in foals at a rate similar to that in ruminant species. In a recently published study of the maturation of renal function in full term pony foals during the first 10 days post partum, it was shown (using the single injection technique) that the glomerular filtration rate and effective renal plasma flow remain relatively constant throughout the post-natal period (Holdstock et al., 1998). This implies that the neonatal foal, like the calf, has relatively mature renal function compared with neonates of most other species. The hydration state of newborn animals would affect renal function (GFR). Even though renal function is immature in neonatal, particularly newborn, animals, it has the capacity adequate to meet physiological requirements. However, when lipid-soluble drugs are administered to neonatal animals, the combined effect of slow hepatic microsomal associated metabolic reactions (oxidation and glucuronide conjugation) and relatively inefficient renal excretion mechanisms considerably decreases the rate of elimination of the parent drugs and their polar metabolites. Urinary pH is acidic in neonates of all species; this would favor renal tubular reabsorption and extend the half-life of drugs that are weak organic acids and of sufficient lipid solubility to be reabsorbed by passive diffusion (e.g., most sulfonamides).

The pharmacokinetic parameters describing the disposition of gentamicin (4 mg/kg, IV) were determined in foals of various ages (12–24 hours, 5, 10, 15 and 30 days) and in mares (Cummings et al., 1990). The apparent volume of distribution of the aminoglycoside did not change significantly with age of the foals, but was approximately twofold larger than in mares. Since the distribution of gentamicin is virtually restricted to the extracellular fluid (ECF), it could be concluded that ECF volume is larger in young foals than in adult horses. Gentamicin is eliminated solely by glomerular filtration. The systemic clearance of gentamicin in 1-day-old foals is similar to that in adult horses; this indicates that glomerular filtration is well developed in newborn foals. Because of the larger volume of distribution and unchanged systemic clearance, the half-life of gentamicin in newborn foals is twice as long as in adult horses, while in foals between 5 and 15 days of age, it is approximately 1.5 times the half-life in adult horses. The pattern of age-related changes in the disposition of gentamicin in calves (Clarke et al., 1985) is similar to that in foals (Table 4.16).

Table 4.16. Age-related changes in the disposition of gentamicin in foals and calves.

Age (days)	$V_{d(ss)}$ (mL/kg)	Cl_B (mL/min \times kg)	$t_{1/2}$ (min)
Foals			
1	307 \pm 30	1.75 \pm 0.47	127 \pm 23
5	350 \pm 66	2.98 \pm 1.48	90 \pm 32
10	344 \pm 95	2.60 \pm 0.96	101 \pm 33
15	325 \pm 48	2.40 \pm 0.87	106 \pm 33
Mares	156 \pm 22	1.69 \pm 0.65	65 \pm 55
Calves			
1	376 \pm 41	1.92 \pm 0.43	149 \pm 38
5	385 \pm 44	2.44 \pm 0.34	119 \pm 20
10	323 \pm 20	2.02 \pm 0.27	118 \pm 13
15	311 \pm 29	2.10 \pm 0.32	111 \pm 8.5
Cows	129 \pm 17	1.29 \pm 0.26	76 \pm 11

The disposition of gentamicin differs significantly between newborn (4 to 12 h of age at the time of dosing) and 42-day-old piglets (Giroux et al., 1995). The age-related pattern of changes in piglets is consistent with that in foals and calves. As the neonate matures, the apparent volume of distribution decreases, systemic clearance increases and the half-life of gentamicin becomes shorter. The average half-life of gentamicin is 5.2 h in newborn piglets, and 3.8 h, 3.5 h, and 2.7 h in 4-, 6-, and 10-week-old piglets, respectively. In a study of the pharmacokinetics of amikacin in critically ill full term foals ranging in age from 2 to 12 days, the systemic clearance of the aminoglycoside was lower and the half-life was considerably prolonged in uremic compared with non-uremic foals (Adland-Davenport et al., 1990). Renal excretion mechanisms appear to mature within the first two weeks after birth in ruminant species, horses and pigs, whereas their maturation in dogs may take 4 to 6 weeks.

The half-life of ceftriaxone, a third-generation cephalosporin that distributes widely in body fluids, penetrates the blood-brain barrier and is eliminated by biliary rather than renal excretion, is twofold longer in 2- to 12-day-old foals (Ringger et al., 1998) than in adult horses (Ringger et al., 1996). The longer half-life of the drug could be attributed to the larger volume of extracellular fluid in the neonatal foals. The average half-life of erythromycin, administered IV as erythromycin gluceptate, in Shetland-cross foals of various ages (from 1 to 12 weeks old) is the same (1 h) as in mares. It is likely that biliary and renal excretion mech-

anisms mature at the same rate in neonatal animals of any species, while hepatic formation of conjugates controls the rate of their excretion in bile and/or urine.

Preference for Bactericidal Drugs

There are certain bacterial infections for which treatment with an antimicrobial agent or combination of agents that produces a bactericidal effect is required. They include meningitis, prostatitis, neonatal septicemia and infections in immunocompromised animals. The immunodeficient state of the neonatal (especially newborn) animal combined with the decreased ability to eliminate (metabolize and excrete) drugs make it preferable to select antimicrobial agents that have a bactericidal action and a wide margin of safety. Such drugs include penicillins, amoxicillin or ticarcillin combined with clavulanic acid, trimethoprim-sulfonamide combinations, and most of the cephalosporins. In the case of infections caused by microorganisms of unpredictable susceptibility (*E. coli*, *Klebsiella*, *Proteus* and *Salmonella* spp., and coagulase-positive staphylococci), the choice of antimicrobial agent and its dosing rate should be based upon in vitro susceptibility, preferably MIC. In the treatment of meningitis, the attainment of effective concentrations in cerebrospinal fluid, which requires penetration of the blood-CSF barrier, is required. A parenteral preparation of the antimicrobial agent selected (often cefotaxime) is administered by slow intravenous injection. In the treatment of prostatitis, relatively high systemic availability of an orally administered drug, penetration of the blood-prostatic fluid barrier and bactericidal action in an acidic environment (pH 6.4) are requirements. Either difloxacin or trimethoprim-sulfadiazine combination may be used and a 12 hour dosage interval applied.

Certain bactericidal drugs used concurrently may act synergistically. Ticarcillin or ceftazidime (or cefoperazone) and gentamicin (or amikacin) act synergistically against *Pseudomonas aeruginosa* provided they attain effective concentrations at the site of infection. Other drugs may be used concurrently to broaden the spectrum of antibacterial activity. In mixed aerobic and anaerobic infections in dogs and cats, the concurrent use of amoxicillin-clavulanate combination, when the primary aerobe is Gram-positive, or enrofloxacin, when the primary aerobe is Gram-negative, and metronidazole (or clindamycin, which is bacteriostatic and time-dependent) may represent the treatment of choice. In horses, the concurrent use of penicillin G,

when the primary aerobe is Gram-positive, or gentamicin (or amikacin), when the primary aerobe is Gram-negative, and metronidazole is indicated.

The concurrent use of a bacteriostatic drug and a bactericidal drug, especially a beta-lactam antibiotic, generally results in antagonism. Nonetheless, the concurrent use of rifampin and erythromycin, both drugs administered orally, is the treatment of choice for *Rhodococcus equi* pneumonia in foals. Rifampin and a tetracycline (doxycycline or minocycline in dogs; oxytetracycline in horses) used concurrently provide enhanced clinical efficacy in *Brucella* infections. Rifampin should always be used in conjunction with another antimicrobial agent, selected on the basis of providing either a synergistic action or enhanced clinical efficacy, to reduce the development of mutants resistant to rifampin. Because of the unique action of rifampin, which is inhibition of bacterial RNA polymerase, cross-resistance between this drug and other antimicrobial agents does not occur.

Bibliography

- Abdullah AS, Baggot JD. 1984. Influence of *Escherichia coli* endotoxin-induced fever on pharmacokinetics of imidocarb in dogs and goats. *Am J Vet Res* 45:2645.
- Abdullah AS, Baggot JD. 1986. Influence of induced disease states on the disposition kinetics of imidocarb in goats. *J Vet Pharm Ther* 9:192.
- Adamson PJW, et al. 1991. Influence of age on the disposition kinetics of chloramphenicol in equine neonates. *Am J Vet Res* 52:426.
- Adland-Davenport P, et al. 1990. Pharmacokinetics of amikacin in critically ill neonatal foals treated for presumed or confirmed sepsis. *Equine Vet J* 22:18.
- Aktas M, et al. 1995. Disposition of creatine kinase activity in dog plasma following intravenous and intramuscular injection of skeletal muscle homogenates. *J Vet Pharm Ther* 18:1.
- Baggot JD. 1977. Principles of Drug Disposition in Domestic Animals: The Basis of Veterinary Clinical Pharmacology. Philadelphia: WB Saunders.
- Baggot JD. 1980. Distribution of antimicrobial agents in normal and diseased animals. *J Am Vet Med Assoc* 176:1085.
- Baggot JD, et al. 1990. Oral dosage of penicillin V in adult horses and foals. *Equine Vet J* 22:290.
- Baggot JD, et al. 1988a. Clinical pharmacokinetics of metronidazole in horses. *J Vet Pharm Ther* 11:417.
- Baggot JD, et al. 1988b. Bioavailability and disposition kinetics of amoxicillin in neonatal foals. *Equine Vet J* 20:125.
- Baggot JD, Short CR. 1984. Drug disposition in neonatal animals, with particular reference to the foal. *Equine Vet J* 16:364.
- Banting A de L, Baggot JD. 1996. Comparison of the pharmacokinetics and local tolerance of three injectable oxytetracycline formulations in pigs. *J Vet Pharm Ther* 19:50.
- Banting A de L, Tranquart F. 1991. Echography as a tool in clinical pharmacology. *Acta Vet Scand, Suppl.* 87:215.
- Benet LZ, Galeazzi RL. 1979. Noncompartmental determination of the steady-state volume of distribution. *J Pharm Sci* 68:1071.
- Bevill RF, et al. 1977. Disposition of sulfonamides in food-producing animals. IV: Pharmacokinetics of sulfamethazine in cattle following administration of an intravenous dose and three oral dosage forms. *J Pharm Sci* 66:619.
- Brown SA, et al. 1986. Dose-dependent pharmacokinetics of gentamicin in sheep. *Am J Vet Res* 47:789.
- Brown SA, et al. 1996. Effects of age on the pharmacokinetics of single dose ceftiofur sodium administered intramuscularly or intravenously to cattle. *J Vet Pharm Ther* 19:32.
- Browne TR, et al. 1990. New pharmacokinetic methods. 111: Two simple tests for "deep pool effect". *J Clin Pharm* 30:680.
- Buonpane NA, et al. 1988. Serum concentrations and pharmacokinetics of chloramphenicol in foals after a single oral dose. *Equine Vet J* 20:59.
- Calder WA. 1984. Size, Function and Life History. Cambridge, MA: Harvard University Press.
- Clarke CR, et al. 1985. Pharmacokinetics of gentamicin in the calf: Developmental changes. *Am J Vet Res* 46:2461.
- Craig W. 1993. Pharmacodynamics of antimicrobial agents as a basis for determining dosage regimens. *Eur J Clin Microbiol Infect Dis* 1 (Suppl):6.
- Cummings LE, et al. 1990. Pharmacokinetics of gentamicin in newborn to 30-day-old foals. *Am J Vet Res* 51:1988.
- Dowling PM, Russell AM. 2000. Pharmacokinetics of a long-acting oxytetracycline-polyethylene glycol formulation in horses. *J Vet Pharm Ther* 23: 107.
- Duffee NE, et al. 1997. The pharmacokinetics of cefadroxil over a range of oral doses and animal ages in the foal. *J Vet Pharm Ther* 20:427.
- Ensink JM, et al. 1994. Oral bioavailability of pivampicillin in foals at different ages. *Vet Quart* 16:S113.
- Firth EC, et al. 1986. Effect of the injection site on the pharmacokinetics of procaine penicillin G in horses. *Am J Vet Res* 47:2380.
- Frazier DL, Riviere JE. 1987. Gentamicin dosing strategies for dogs with subclinical renal dysfunction. *Antimicrob Agents Chemother* 31:1929.
- Frazier DL, et al. 1988. Gentamicin pharmacokinetics and nephrotoxicity in naturally acquired and experimentally induced disease in dogs. *J Am Vet Med Assoc* 192:57.
- Gilman JM, et al. 1987. Plasma concentration of gentamicin after intramuscular or subcutaneous administration to horses. *J Vet Pharm Ther* 10:101.
- Giroux D, et al. 1995. Gentamicin pharmacokinetics in newborn and 42-day-old male piglets. *J Vet Pharm Ther* 18:407.
- Grondel JL, et al. 1989. Comparative pharmacokinetics of oxytetracycline in rainbow trout (*Salmo gairdneri*) and

- African catfish (*Clarias gariepinus*). J Vet Pharm Ther 12:157.
- Henry MM, et al. 1992. Pharmacokinetics of cephadrine in neonatal foals after single oral dosing. Equine Vet J 24:242.
- Holdstock NB, et al. 1998. Glomerular filtration rate, effective renal plasma flow, blood pressure and pulse rate in the equine neonate during the first 10 days post partum. Equine Vet J 30:335.
- Huffman EM, et al. 1981. Serum chloramphenicol concentrations in preruminant calves: A comparison of two formulations dosed orally. J Vet Pharm Ther 4:225.
- International Commission of Radiological Protection. 1975. Report of the Task Group on Reference Man, 1st ed. Oxford: Pergamon.
- Jaglan PS, et al. 1990. Liquid chromatographic determination of desfuroylceftiofur metabolite of ceftiofur as residue in cattle plasma. J Assoc Offic Analyt Chemists 73:26.
- Kaartinen L, et al. 1995. Pharmacokinetics of enrofloxacin after single intravenous, intramuscular and subcutaneous injections in lactating cows. J Vet Pharm Ther 18: 357.
- Kaartinen L, et al. 1997. Pharmacokinetics of enrofloxacin in newborn and one-week-old calves. J Vet Pharm Ther 20:479.
- Kinoshita T, et al. 1995. Impact of age-related alteration of plasma α_1 -acid glycoprotein concentration on erythromycin pharmacokinetics in pigs. Am J Vet Res 56:362.
- Korsrud GO, et al. 1993. Depletion of intramuscularly and subcutaneously injected procaine penicillin G from tissues and plasma of yearling beef steers. Canadian J Vet Res 57: 223.
- Love DN, et al. 1981. Serum levels of amoxicillin following its oral administration to Thoroughbred foals. Equine Vet J 13:53.
- Marshall AB, Palmer GH. 1980. Injection sites and drug bioavailability. In: Van Miert ASJPAM, et al. (eds). Trends in Veterinary Pharmacology and Toxicology. Amsterdam: Elsevier, p54.
- Martinez MN, Berson MR. 1998. Bioavailability/bioequivalence assessments. In: Hardee GE, Baggot JD (eds) Development and Formulation of Veterinary Dosage Forms, 2nd ed. New York: Marcel Dekker, p 429.
- Matthews CA, et al. 1975. External form and internal anatomy of Holsteins and Jerseys. J Dairy Sci 58:1453.
- Mengellers MJB, et al. 1995. Pharmacokinetics of sulfadimethoxine and sulfamethoxazole in combination with trimethoprim after intravenous administration to healthy and pneumonic pigs. J Vet Pharm Ther 18:243.
- Neff-Davis, C, et al. 1975. Comparative body composition of the dog and goat. Am J. Vet Res 36:309.
- Nielsen P, Rasmussen F. 1976. Influence of age on half-life of trimethoprim and sulphadoxine in goats. Acta Pharm Toxicol 38:113.
- Nielsen P. 1997. The influence of feed on the oral bioavailability of antibiotics/chemotherapeutics in pigs. J Vet Pharm Ther 20 (Suppl 1):30.
- Nouws JFM, et al. 1993. Pharmacokinetics of sulphadiazine and trimethoprim in carp (*Cyprinus carpio* L.) acclimated at two different temperatures. J Vet Pharm Ther 16:110.
- Nouws JFM, et al. 1982. Comparative plasma ampicillin levels and bioavailability of five parenteral ampicillin formulations in ruminant calves. Vet Quart 4:62.
- Nouws JFM, Vree TB. 1983. Effect of injection site on the bioavailability of an oxytetracycline formulation in ruminant calves. Vet Quart 5:165.
- Olson SC, et al. 1998. In vitro metabolism of ceftiofur in bovine tissues. J Vet Pharm Ther 21:112.
- Pennington JE, et al. 1975. Gentamicin sulfate pharmacokinetics: lower levels of gentamicin in blood during fever. J Infect Dis 132:270.
- Rasmussen F. 1966. Studies on the Mammary Excretion and Absorption of Drugs. Copenhagen: Carl Fr. Mortensen.
- Reiche R, et al. 1980. Pharmacokinetics of chloramphenicol in calves during the first weeks of life. J Vet Pharm Ther 3:95.
- Reiche R. 1983. Drug disposition in the newborn. In: Ruckebusch Y, et al. (ed). Veterinary Pharmacology and Toxicology. Lancaster, U.K.: MTP Press, p 49.
- Ringger NC, et al. 1998. Pharmacokinetics of ceftriaxone in neonatal foals. Equine Vet J 30:163.
- Ringger NC, et al. 1996. Pharmacokinetics of ceftriaxone in healthy horses. Equine Vet J 28:476.
- Rutgers LJE, et al. 1980. Effect of the injection site on the bioavailability of amoxycillin trihydrate in dairy cows. J Vet Pharm Ther 3:125.
- Shoaf SE, et al. 1987. The development of hepatic drug-metabolizing enzyme activity in the neonatal calf and its effect on drug disposition. Drug Metab Dispos 15:676.
- Smith CM, et al. 1988. Effects of halothane anesthesia on the clearance of gentamicin sulfate in horses. Am J Vet Res 49:19.
- Spector R, Lorenzo AV. 1974. Inhibition of penicillin transport from the cerebrospinal fluid after intracisternal inoculation of bacteria. J Clin Invest 54: 316.
- Toutain P-L, et al. 1995. A non-invasive and quantitative method for the study of tissue injury caused by intramuscular injection of drugs in horses. J Vet Pharm Ther 18:226.
- Webb AI, Weaver BMQ. 1979. Body composition of the horse. Equine Vet J. 11:39.
- Wilson RC, et al. 1989. Bioavailability of gentamicin in dogs after intramuscular or subcutaneous injections. Am J Vet Res 50:1748.
- Xia W, et al. 1983. Comparison of pharmacokinetic parameters for two oxytetracycline preparations in pigs. J Vet Pharm Ther 6:113.
- Zeng Z, Fung K. 1997. Effects of experimentally induced *Escherichia coli* infection on the pharmacokinetics of enrofloxacin in pigs. J Vet Pharm Ther 20 (Suppl 1):39.

The Pharmacokinetic-Pharmacodynamic (PK/PD) Relationship of Antimicrobial Agents

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Antimicrobial pharmacokinetic (PK)/pharmacodynamic (PD) relationships reflect a correlation between the drug concentration in the blood, the concentration of the biologically active drug at the site of the infection, and the microbial or clinical outcome (Levison, 2004). The PK component (Chapter 4) describes the processing of the drug by the host (absorption, distribution, metabolism, and elimination). The PD component describes the effect of the drug on the bacterial pathogen(s). By identifying an association between pharmacokinetics and pharmacodynamics for a specific host-drug-microbe combination, the PK/PD approach provides a valuable guide for estimating the doses and dosage regimens that can optimize the bacteriological or clinical outcome. However, PK/PD indices cannot be used for assessing product clinical effectiveness, nor should they be used for predicting therapeutic effectiveness of antimicrobial compounds.

While there are a number of factors that contribute to the PK/PD index [e.g., the *in vitro* minimum inhibitory concentration (MIC) of the drug, its post-antibiotic effects (PAE), and sub-MIC effects], there are also several factors that the traditional PK/PD approach does not describe. For example, the *in vitro* MIC, which is the basis for the PD component of these indices, does not provide information on time to kill, time to maximum kill, log change within a fixed time, or the maximum reduction in viable bacterial counts (MacGowan and Bowker, 2002). There are also many other *in vivo* factors that influence antimicrobial effectiveness (e.g., anti-inflammatory effects, presence of bacterial biofilms, the drug's ability to interfere with bacterial colonization on epithelial surfaces, and the

influence of the drug on toxin production and release). These additional considerations are difficult to assess in the absence of actual clinical data. Furthermore, plasma drug concentrations, the primary component for building the PK portion of the PK/PD index, do not necessarily reflect a compound's ability to diffuse into the site of infection and into the bacterial cell.

There are seven distinct factors affecting the relationship of a drug within a dosage form and the success of the anti-infective treatment (Table 5.1). In this chapter, each of these variables are discussed, as it influences the value and validity of the PK/PD approach. Tools currently available for assessing the relationships between PK and PD of antimicrobials and the potential use of this information for optimizing antimicrobial dosage regimens are also considered. Dose optimization not only insures the most cost-effective use of these agents but also contributes, on a long-term basis, to the effectiveness of our current and future antimicrobial arsenal.

Understanding the Drug Response

The first step in understanding PK/PD relationships is to identify the mechanisms through which drug-pathogen interactions occur (Table 5.4). These mechanisms of action dictate the PD characteristics of the drug, including whether it will result in static or cidal activity, its rate of kill, the ability to retain effectiveness after systemic drug concentrations have dropped below the MIC of the pathogen, and its ability to act on bacteria that are in a stationary growth phase.

Table 5.1. Factors that can influence the therapeutic effect of an antimicrobial agent.

1. The **pharmacokinetics** of the drug. This includes the PK of the active pharmaceutical ingredient (API) and its release from the formulated dosage form.
2. The **site and nature** of the infectious disease process.
3. The **clinical objective** of the therapy, which may differ in food versus companion animal species.
4. The integrity of the **host immune system** and its ability to interact with the bacterial pathogen.
5. The influence of the antimicrobial agent on the **disease process**. This includes:
 - a. Its action and mechanism of action (e.g., static versus cidal, inhibition of cell wall production versus protein synthesis).
 - b. The potency of the drug against the targeted pathogen, which is generally expressed in terms of the *in vitro* MIC.
 - c. The activity of the antimicrobial agent against the pathogen as a function of drug concentration and duration of exposure.
 - d. The relationship between the rates at which the drug kills the bacterial pathogen versus the rate of the pathogen's proliferation.
 - e. The virulence factors associated with the pathogen. This includes the formation of toxins and toxic metabolites, and the effect that these toxic substances have on the host immune system.
 - f. The ability of the drug to alter the bacterium's adherence and colonization to epithelial surfaces.
 - g. The effect the drug has on planktonic cells versus biofilm imbedded organisms, and on the logarithmic versus stationary growth phases of the microbes.
6. The potential for the pathogen to form **biofilms (or persister cells)** that protect the microbe against both the host defenses and the effects of the antimicrobial agent.
7. The potential for the development of **resistant strains**, which can be influenced by such factors as:
 - a. Sub-optimal drug concentrations at the site of infection.
 - b. Microbial reproduction rate.
 - c. Concentration of pathogen at the site of infection.
 - d. Mechanisms of resistance.
 - e. The heterogeneity of the pathogen population.

General Pharmacokinetic Considerations

For any antimicrobial agent, the primary target is the invading pathogen. Thus, the objective for any therapeutic intervention is to ensure that adequate active drug concentrations reach the infection site. Although bacterial pathogens may be found in blood during dissemination (bacteremia), the site of a bacterial infection is seldom in the vascular bed. Instead, the primary site of bacterial infections is nearly always located in tissues requiring a PK step of drug distribution.

Drug kinetics may be represented as a compartmental model (Figure 5.1). In this model, the drug enters the body via the blood (i.e., the "central compartment"). This implies either oral administration of a

drug that is systemically absorbed or parenteral administration. From the blood, the drug is delivered to the remainder of the body either very rapidly in well-perfused tissues belonging to the central compartment (liver, kidney, lungs) or more slowly to other tissues (the so-called "peripheral compartment"). While some drug loss within peripheral tissues may occur (e.g., local drug metabolism), it is expected to be minimal compared to the magnitude of drug elimination via the kidneys, liver, and possibly lungs (i.e., organs located in the central compartment). Therefore, the clearance component of the model has the drug exiting the body directly and exclusively from the central compartment. A complete review of the bioavailability, disposition, and elimination of antimicrobial agents may be found in Chapter 4.

PK metrics used for the PK/PD approach are not appropriate for compounds administered directly to the site of action. These include orally administered drugs that are not systemically absorbed but are intended to treat local GI infections, intramammary administration, some otic and ophthalmic products, and inhaled antimicrobial compounds.

Drug Transport to the Site of Infection

For treatments requiring systemic administration, the ability of the drug to reach the pathogens requires the following processes: (1) drug dissolution and absorption (membrane permeation); (2) drug bioconversion (if there are active metabolites or if the active pharmaceutical ingredient is a pro-drug); (3) transport of the active drug from the blood to the site of infection; and (4) movement of free drug into the bacterial cell and the targeted bacterial structure or organelle. The relationship between drug concentrations in the serum versus the concentration at the site of the infection (i.e., the biophase) is presented in Figure 5.2.

The site of infection is a critical consideration in the use of plasma concentrations to predict the clinical effectiveness of an antimicrobial agent. From high to low correspondence, the relationship between drug concentration in the plasma and at the site of infection can be ranked as follows (Craig, 2004): (1) serum; (2) interstitial fluids; (3) fluid collections (sinusitis, acute otitis media); (4) abscesses; (5) epithelial lining fluid; (6) cerebrospinal fluid (CSF) and prostate; (7) intracellular milieu; and (8) urine.

Although plasma free drug concentrations do not necessarily translate directly into concentrations at the

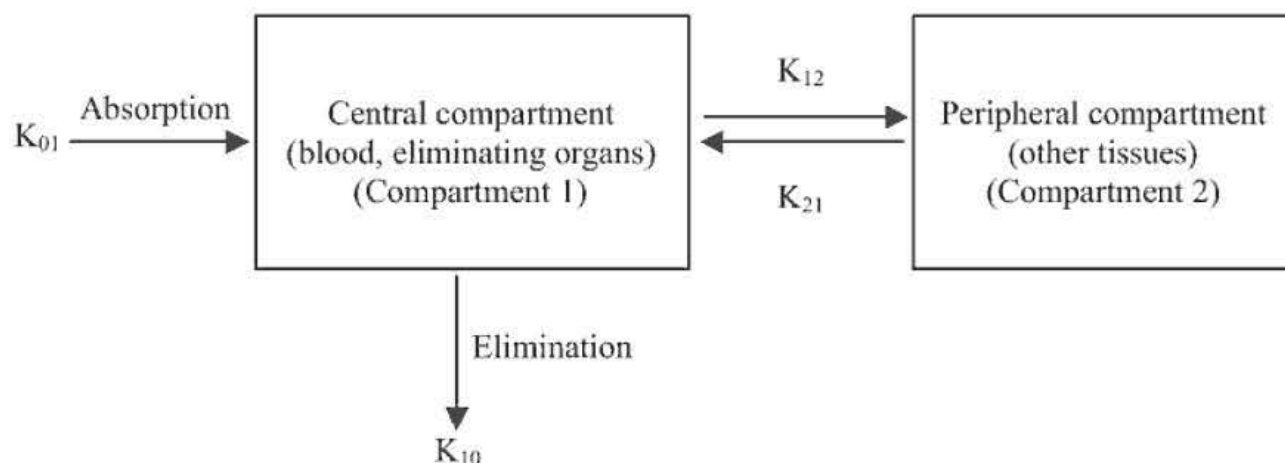


Figure 5.1. A compartmental pharmacokinetic model describing the stochastic movement of molecules through the body.

site of the infection, for extracellular infections, concentrations at the infection site are usually represented (at least proportionally) by plasma free drug concentrations. Therefore, PK/PD relationships should be based upon free drug concentrations (Liu et al., 2005; Mueller et al., 2004; Drusano, 2004). An example of the relationship between free versus total plasma drug concentrations and free drug concentration in the extracellular fluids of muscle and lung is provided in Figure 5.3.

There are also examples where markedly different plasma-biophase concentrations are observed. A case in point are the penicillins and cephalosporins which, unlike the fluoroquinolones (Cazzola et al., 2002), reach only marginal concentrations in pulmonary secretions (Agero et al., 1998). For the aminoglycoside tobramycin, high peak serum concentrations are necessary to obtain microbiologically active concentrations at the alveolar level, and the epithelial lining fluid constitutes a deep compartment for this compound (Carcas et al., 1999).

The opposite relationship between blood and tissue is seen with macrolides, ketolides, and azalides, where pulmonary tissue fluid concentrations far exceed concentrations observed in the plasma. This apparent inconsistency likely reflects the uptake of these drugs into tissues (Honeybourne et al., 1994). While some claim that the very high tissue concentrations are attributable to partitioning of drug into leukocytes (Scorneaux and Shryock, 1999), others claim that these concentrations reflect binding to functional groups on biological membranes, such as binding to phosphatidyl serine residues (Yata et al., 1990). Regardless of the mechanism by which this accumulation occurs, the PK/PD relationships for macrolides, ketolides and azalides should not

be based solely upon in vitro concentration-effect data. For example, for the ketolide telithromycin, an AUC/MIC ratio of 3.375 (i.e., effective concentrations equal to only 15% of the in vitro MIC) was found to adequately predict 90% eradication of bacterial respiratory pathogens in human patients with intact immune function (Drusano and Preston, 2002).

Occasionally, penetration in healthy tissue does not reflect the penetration that occurs in diseased tissues. Because the volume of the infection site is generally small as compared to the rest of the systemic volume of distribution, these changes are rarely predicted solely from blood concentration data. Reasons for this difference in drug distribution into healthy versus infected tissue can be multifold. For example, the increase in blood flow that may occur during an acute inflammatory response can increase the rate of drug exchange between the blood and the inflamed tissue (Ryan, 1993). This has been recently demonstrated for ciprofloxacin in humans, where warming of a lower extremity increased the microcirculatory blood flow to approximately three to four times baseline. The ratio of the C_{\max} of unbound ciprofloxacin for the warmed thigh to the C_{\max} of the non-warmed thigh was higher by a factor of 2.1 ± 0.90 (Joukhadar et al., 2005). Conversely, during chronic inflammation, physical alteration at the infection site may impair drug penetration. In these situations, drug concentrations available within an infection site may decrease due to a decrease in the diffusivity of the drug through the infection-induced exudates or changes in the diffusion distance that occurs with swelling (i.e., the distance between the infected tissue and the blood supply). As discussed

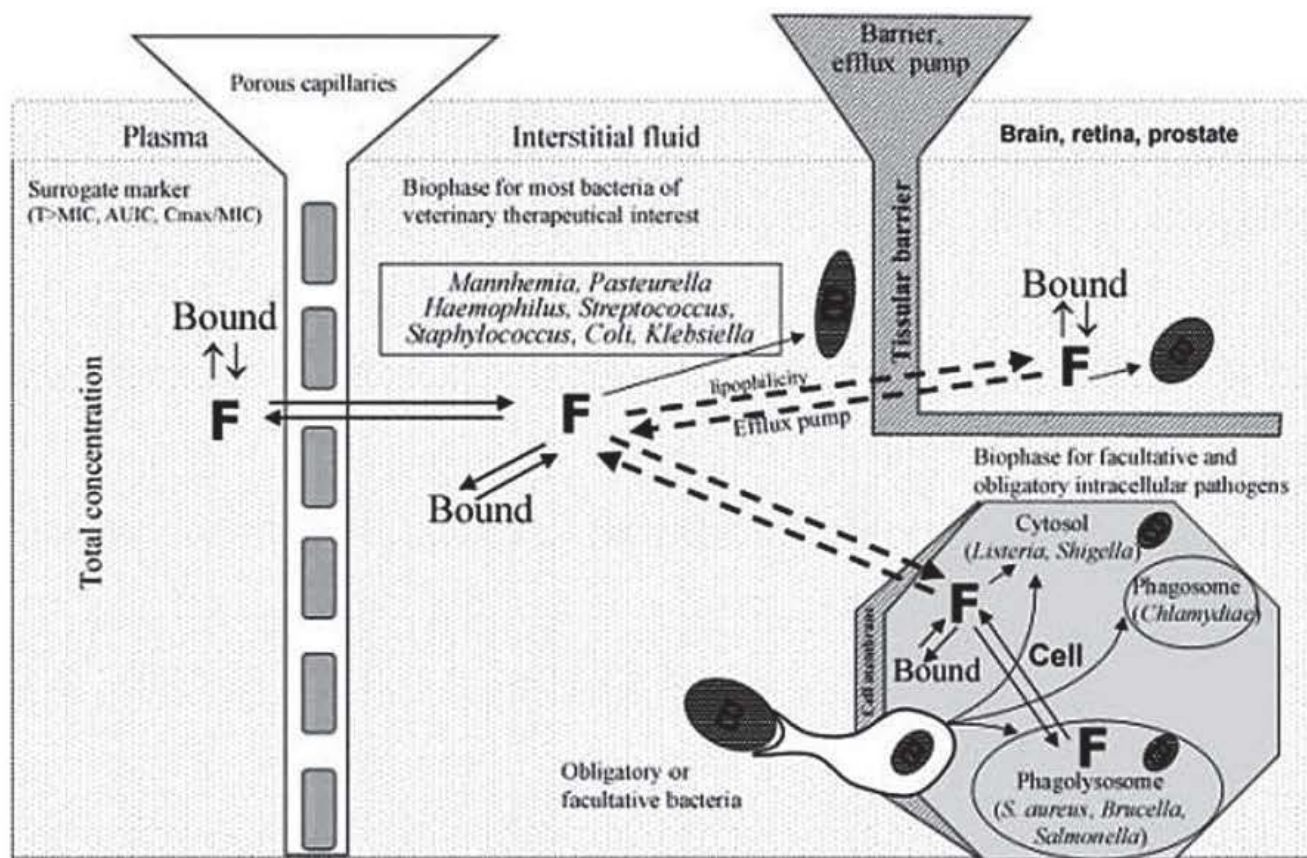


Figure 5.2. Antibiotic access to the bacterial biophase. Most bacteria (B) are located in the extracellular fluid (plasma and interstitial fluids). Unbound drug (F) circulating in plasma is the only fraction which can access the interstitial fluid through porous capillaries to combat infection caused by an extracellular pathogen. A drug will have antibacterial activity if its unbound concentration exceeds the MIC of the pathogen. Some tissues have permeability limitations at the capillary level and/or possess an efflux pump. This impedes accumulation of drugs in the tissue (e.g., blood-brain barrier) and only lipophilic drugs can cross such barriers (e.g., quinolones). The blood perfusion rate can also be a limiting factor (clot, abscess). Some bacteria are located within cells (facultative or obligatory intracellular pathogens). Inside a given cell, different antibiotic concentrations are possible in different locations (cytosol, phagosome, and phagolysosome). Macrolides, for example, are trapped in phagolysosomes which have a low pH (about 4–5), resulting in a high “total cell” concentration. However, as the antibacterial potency of macrolides is pH-dependent (low or no activity at acidic pH), a high local concentration is not synonymous with high activity. From Toutain (2002) with permission.

later in this chapter, the presence of biofilms may further impair drug access to the pathogen or the susceptibility of the pathogen to the antimicrobial agent.

Measurements of total drug concentrations in normal healthy tissue may not accurately reflect the concentration of active drug available in infected or inflamed tissues. For example, mastitis generally results in an increase in the pH of milk (the pH of milk is normally 6.6 to 6.8, but can go as high as 7), although rare instances of a decrease in milk pH have been reported. With these changes in pH, there can be changes in the

ratio of ionized to nonionized drug. Therefore, total drug concentrations in normal healthy quarters may not reflect active drug concentrations in inflamed and infected udders (Gips and Soback, 1999). If the non-ionized drug does not freely distribute between the blood and the infection site, compounds may be ineffective in treating the clinical infection, despite excellent in vitro susceptibility and excellent apparent partitioning into the tissues of normal healthy subjects.

Similarly, the presence of antibiotics in the urine is correlated with bacteriological cure for uncomplicated

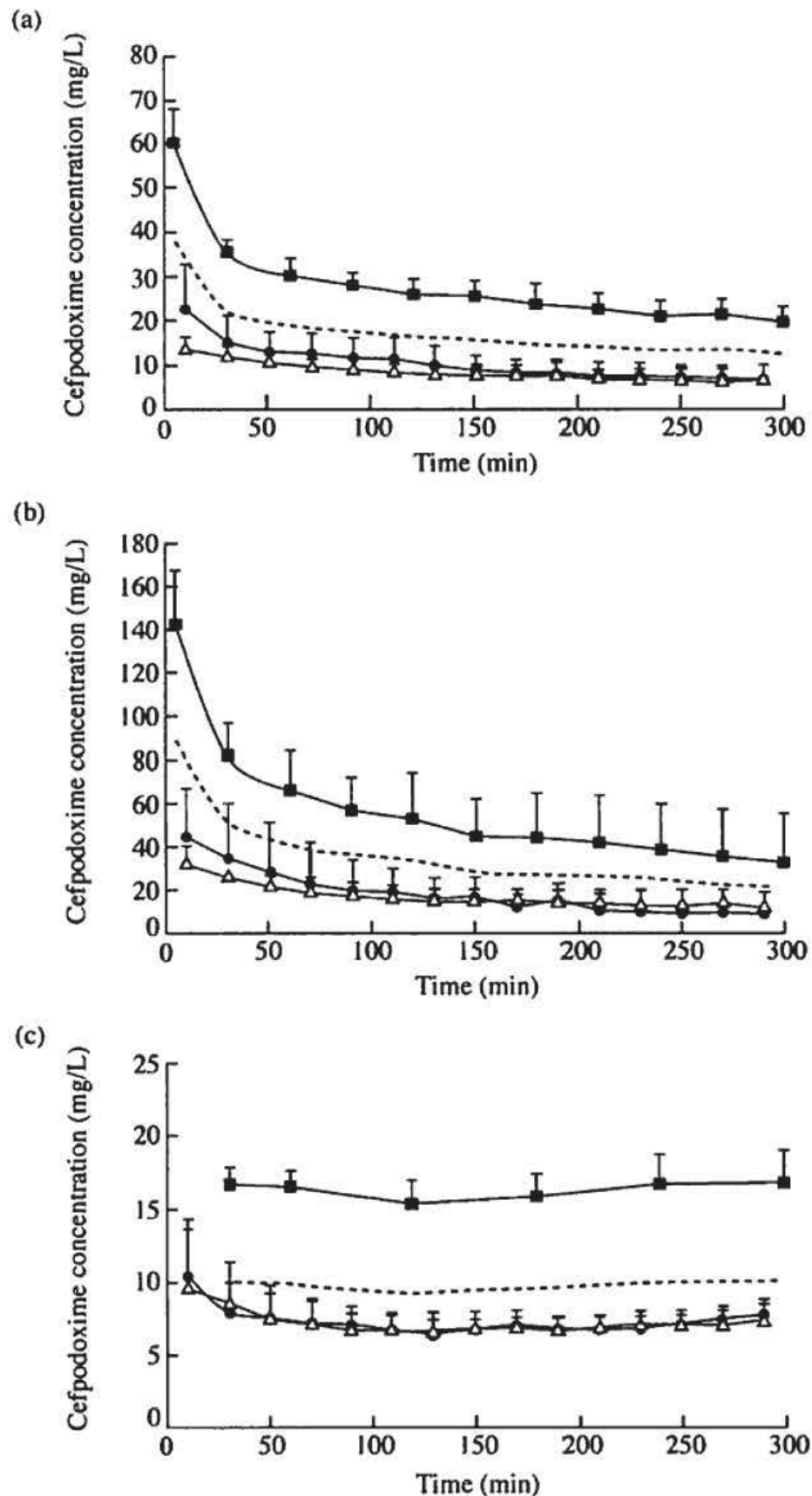


Figure 5.3. Total plasma (closed squares), free lung (closed circles), free muscle (open triangles) and calculated free plasma (dotted line) concentrations of cefpodoxime in male Wistar rats determined by microdialysis following an iv dose of (a) 10 mg/kg cefpodoxime ($n=6$), (b) 20 mg/kg cefpodoxime ($n=6$), or (c) at steady state during continuous infusion of 260 $\mu\text{g/h}$ after a loading dose of 6 mg/kg cefpodoxime ($n=6$). Values are mean \pm s.d. From Liu et al (2002) with permission.

Table 5.2. Variables that can influence the interpretation of tissue drug concentrations across a variety of sampling methods.

	Tissue Ground HPLC	Fluid Sample HPLC	Fluid Sample Bioassay	μDialysis HPLC	μDialysis Bioassay
Blood vs. Tissue	?				
Intracellular vs. Extracellular	?				
Protein binding	?	?	-/?		
Ionized vs. Nonionized	?	?	?	?	?
Stereoisomer	?	?		?	
Metabolites			?		?

HPLC: High pressure liquid chromatography.

μDialysis: Microdialysis.

?: Variable cannot be quantified and therefore can influence study interpretation.

-/? : May or may not have an effect, depending upon the rate of dissociation from the protein.

urinary tract infection (UTI). However, these luminal drug concentrations appear to be virtually ineffective against bacterial growth in the bladder wall (Frimodt-Møller, 2002). In the latter situation, the drug needs to reach the infected tissue via the blood (Frimodt-Møller et al., 1981). In addition, at least in human patients, chronic bladder infections may be attributable to the intracellular invasion of uropathogenic *Escherichia coli* (UPEC). Evidence suggests that UPEC invades bladder epithelial cells where it replicates to form large bacterial inclusions. This may trigger host exfoliation of these infected cells, as well as cytokine production. Prior to completion of cellular sloughing, UPEC emerges from infected cells, forming new contacts with the exposed transitional epithelium. The intracellular phase provides a significant challenge to the effective use of antimicrobials for the treatment of chronic UTI's (Schilling and Hultgren, 2002). This underscores the importance of identifying the location of the pathogen when determining the targeted destination for antimicrobial therapy.

These various points emphasize the important considerations that need to be factored into the interpretation of PK data, including an appreciation of the limitations associated with the various experimental tools. For example, when examining blood or tissue concentrations, results and conclusions may differ if using an HPLC (LC/MS-MS) or microbiological assay method. The former generally does not distinguish between bound versus free compound and may not be able to distinguish between active and inactive enantiomers. On the other hand, microbiological methods measure only active compound, but may not distin-

guish between the parent drug and its active metabolites. This difference may be critical, as the microbiological species used in the assay may be highly susceptible to the active compound and the metabolite, but the pathogen may exhibit a markedly different spectrum of activity (Nolan and Ulmer, 1980).

Even when using techniques such as microdialysis and stereospecific assays, it is difficult to determine if the observed tissue concentrations reflect ionized or nonionized compounds. Table 5.2 provides a list of factors that can influence the results obtained when using the various methods (potential influences are denoted by a question mark). Not included in this table are tissue blister fluids or tissue cage models. A unique problem presented by these methods is the influence of model infection site geometry versus the diffusion of drug (Ryan, 1993). Therefore, the results from these studies present an additional set of variables that should be considered with those in Table 5.2.

Importance of pH Considerations

Only ionized compounds are soluble in an aqueous environment, and compounds must be dissolved in an aqueous environment to be absorbed. However, with the exception of very small molecules that can move past pores in a biological membrane (paracellular transport) or are actively transported into cells, it is only the nonionized drug that can cross into the systemic circulation or into cells and organelles (Martinez and Amidon, 2002). This is a very important factor for antimicrobials which tend to be weak acids (e.g., beta-lactams), weak bases (e.g., macrolides), and zwitterions (e.g., fluoroquinolones).

The influence of pH on antimicrobial activity (in vitro vs. *in vivo*) and drug concentrations (total and nonionized) needs to be seriously considered. For example, pH may increase in the case of bovine mastitis and may decrease in the presence of soft tissue infection. Drugs entering the urine will encounter a markedly different pH from that of the blood. However, the influence of pH is often overlooked. A typical example of this point is that total drug concentrations in the urine are often assumed to represent active drug because protein binding in the urine is negligible. However, if the drug is largely ionized in urine, then only a fraction of the total drug concentration may actually represent biologically active compound.

To demonstrate the influence of pH on the proportion of drug existing in the ionized (I) or nonionized (UI) state, the authors of this chapter conducted an *in silico* experiment. Two conditions were examined: a putative biophase pH drop to 6.5 and an increase to 7.5. For the sake of this evaluation, the pH of the "plasma" compartment (P) was held at 7.0, the drug was a weak acid, and the pKa of the compound was 7.0. For non-septicemic infections, the biophase is located in extravascular spaces (such as extracellular fluids, intracellular space or some biological fluid such as milk, urine, etc.) and is generically described as "tissular" infection (T). To demonstrate the importance of the volume of P relative to that of T, the volume of T was held constant (one unit) while the volume of P was either 100 units, one unit or zero units (where zero units represents impact of pH on I and UI when the potency of a compound is tested under traditional in vitro susceptibility test conditions). For the weak acid, the ratio of UI and I was determined by the Henderson-Hasselbach equation: $\text{pH} = \text{pKa} + \log (C_i/C_u)$, where C_i and C_u are the concentration of ionized (ineffective) and nonionized (effective) drug forms, respectively. It was assumed that the concentrations of I and UI in the plasma compartment (P-I and P-UI respectively) are equal since $\text{pH} = \text{pKa}$, that the concentrations of UI in the tissue compartment (T-UI) are equal to P-UI, and that the concentration of T-I varies as a function of T-UI and the ratio associated with the Henderson-Hasselbach equation. To accommodate different volumes of P, values were estimated in terms of amount of drug (where for $P = 100$ units, amount of drug in T-UI = $0.01 \times$ amount in P-UI, and where $P = 1$, amount of drug in T-UI =

amount in P-UI). The results of this exercise are provided in Figures 5.4a and 5.4b.

The important points to note are that under conditions approximating most real life situations (where the volume of P is substantially greater than the volume of the infection site), changes in pH do not affect blood concentrations of UI, even if there are changes in the total drug concentrations in T. Under equilibrium conditions, changes in local pH do not affect the concentration of UI in the "tissue". The only moiety affected is T-I. As the volume of P approaches that of T, changes in pH begin to influence T-UI, T-I, P-I, and P-UI. From these simulations, it should be clear that when testing "total" drug concentrations in the tissue (T-I + T-UI), one can be led to the erroneous conclusion that there is a substantial change in active drug concentration in the infection site.

In the most extreme theoretical case, $P=0$, there is no buffering from the P compartment, and T-UI and T-I are strictly functions of pH and drug pKa. This is the situation that is seen with most microbiological in vitro susceptibility test procedures. Therefore, these in vitro tests may exaggerate the physiological consequences of alterations in pH at an infection site.

Relating this to PK/PD, assuming that only the UI form has a biological effect, the question is: To what extent does pH influence drug effects in vivo? This effect appears to largely depend upon the rate of exchange between the blood and the infection site and whether or not the infection site represents a well-stirred system. In most tissues, there is a high level of exchange between the blood and the extracellular fluids, and the dispersion of drug within tissular fluids is rapid. In these cases, the system will tend towards conditions represented as a well-stirred system, and we can anticipate that near equilibrium conditions (e.g., that of $P=100$) will exist (Oliver et al., 2001). Therefore, T-UI will equal P-UI. However, as the rate of exchange between infection site and blood decreases, the system begins to deviate from the well-stirred situation. The latter is a concern in restricted tissues (e.g., central nervous system and prostate), poorly perfused infection sites (e.g., foot rot in cattle), and sites with unique kinetic features (e.g., urine and milk). It may also be a problem as bacteria change from free floating, planktonic cells to a component of a biofilm. In these cases, there is an increasing possibility that the concentration of T-UI will not be in equilibrium with P-UI and relationships will begin to more closely resemble the simulation where P is small.

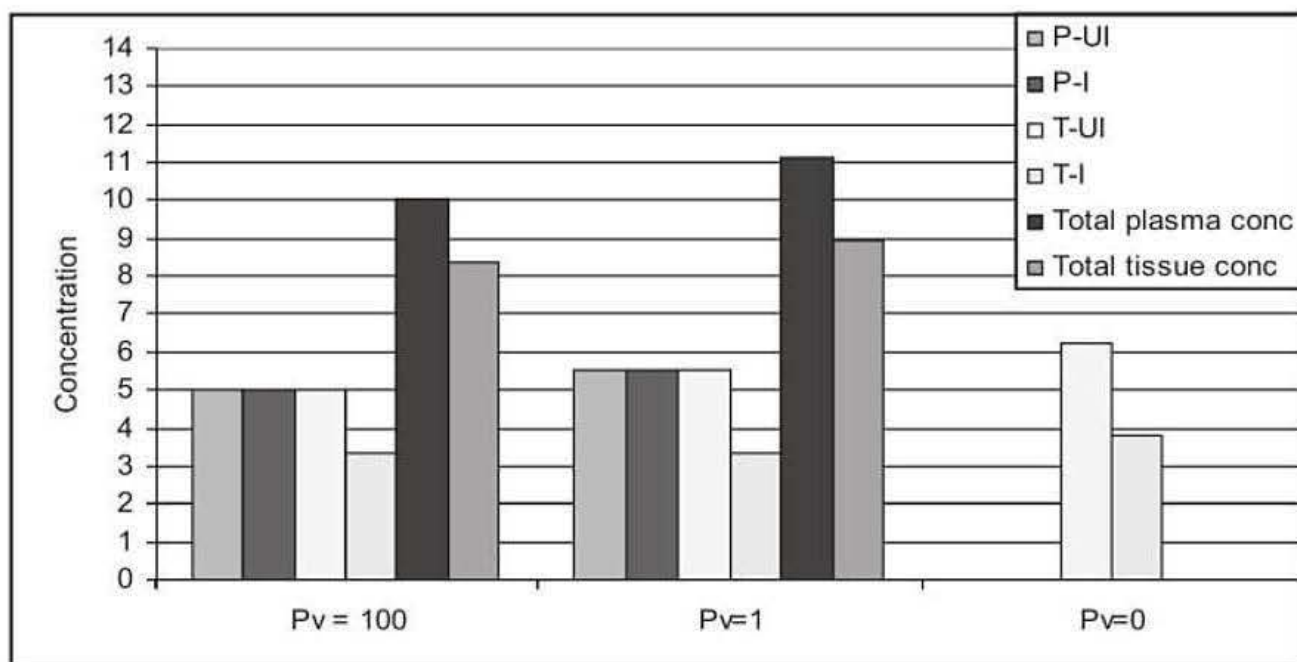


Figure 5.4a. Impact of "central" volume on weak acid nonionized drug concentration in the "tissue" compartment if pH drops to 6.5.

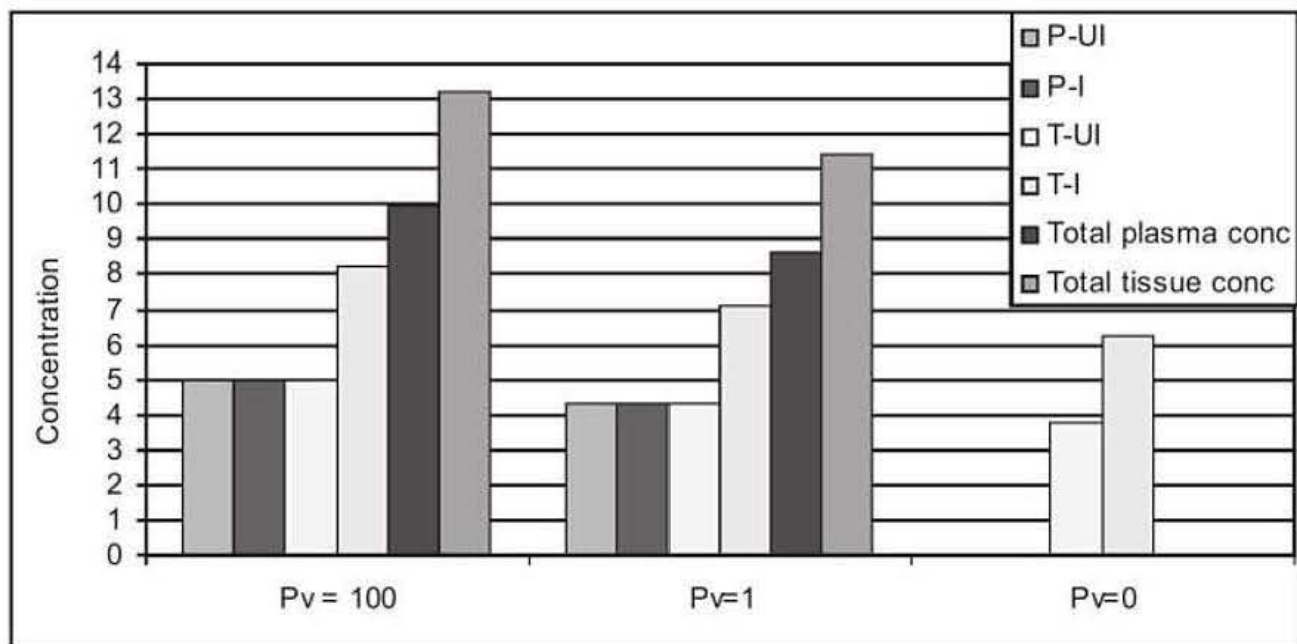


Figure 5.4b. Impact of "central" volume on weak acid nonionized drug concentration in the "tissue" compartment if pH increases to 7.5.

Owing to the lack of buffering capability, we see that weak bases such as macrolides, tending to be ionized at lower pH values, can have a significantly reduced in vitro potency as the pH of the test medium decreases. This is because for weak bases, a decrease in pH will lead to an increase in the proportion of ionized drug and therefore a decrease in the potency (increase in the MIC) when estimated on the basis of total drug concentrations. This is clearly seen in Table 5.3 where the in vitro MIC value for tulathromycin is assessed over a range of pH values. This ionization, at least in part, explains the intracellular accumulation (particularly in neutrophils and macrophages) seen with many macrolides (Carbon, 1998). This pH sensitivity can be problematic under in vitro test conditions, as incubation with CO₂ can lower the pH of the growth medium. More details on the relative contribution of ion-trapping and intracellular binding to the tissue retention of drug can be found elsewhere (Siebert et al., 2004).

Antimicrobial Pharmacodynamics: General Concepts

Successful antimicrobial chemotherapy depends on an identification of the etiological agent(s), selection of the appropriate antimicrobial agent, and the use of an appropriate dosing regimen.

When considering the choice of antimicrobial agent and dosing regimen, the veterinary practitioner needs to consider the pharmacokinetics of the chosen drug in the targeted animal species and the pharmacodynamic indices that drive its clinical effectiveness. For example, penicillins, like all beta-lactam antimicrobials (i.e., penicillins, cephalosporins, carbapenems and monobactams), exhibit time-dependent killing. This infers that maximum clinical effectiveness is achieved by ensuring that the free (i.e., not bound to proteins) serum concentration of the selected beta-lactam exceeds the MIC of the pathogen for the appropriate percentage of the dosing interval. If the pathogen is a Gram-positive organism, the targeted duration is usually $\geq 40\%$ of the dosing interval. In contrast, concentrations of most beta-lactams should exceed the MIC of the pathogen by $\geq 80\%$ of the dosing interval when the infectious agent is a Gram-negative organism.

In other words, for drugs exhibiting time-dependent killing, increasing the concentration of the drug in ex-

Table 5.3. The effect of pH on in vitro tulathromycin activity.

Microorganism ^a	Mean MIC ($\mu\text{g/mL}$) at pH ^b					
	6.5	7.0	7.2	7.4	7.6	8.0
<i>E. coli</i> ATCC 25922	>128	18.4	4.59	2.00	2.00	2.00
<i>E. faecalis</i> ATCC 29212	>128	38.8	12.1	3.48	2.00	2.30
<i>S. aureus</i> ATCC 29213	>128	24.3	8.00	3.03	1.74	2.00

^aQuality control isolates obtained from the American Type Culture Collection (ATCC).

^bStandard testing conditions consistent with CLSI methods were used, except that pH of the culture medium was varied as indicated.

From: US FDA Center for Veterinary Medicine, 2004.

cess of the MIC of the pathogen does not dramatically increase the rate of killing. Rather, the extent of killing is dictated by the duration of time that the bacteria are exposed to the drug.

Conversely, most bactericidal antimicrobial agents that interfere with DNA or RNA synthesis (fluoroquinolones and aminoglycosides) exhibit concentration-dependent killing. In this situation, the rate of killing increases as the drug concentration increases above the MIC of the bacterial pathogen. Thus, antimicrobial agents may be classified as those that exhibit time-dependent killing (all beta-lactams), concentration-dependent killing (fluoroquinolones and aminoglycosides), and those that are generally considered to be bacteriostatic (e.g., tetracyclines, macrolides and lincosamides).

Mouton et al. (2005) published an attempt to standardize the interpretation of these various PK/PD parameters. Some of the basic definitions are as follow:

- **AUC** should be expressed in terms of unbound drug. If multiple dosing regimens are applied, AUC should be measured over a 24-hour dosing interval at steady state. In this regard, it should be noted that for compounds exhibiting linear PK, the AUC over a single dosing interval at steady state ($\text{AUC}_{0-\tau}$) is equal to AUC extrapolated to time infinity ($\text{AUC}_{0-\infty}$) following a single administration.
- **AUC/MIC**. Although sometimes given the dimension of time, because it is measured over a set period of incubation (generally 18 to 24 hours), this ratio can be more conveniently expressed as a dimensionless value.
- **T>MIC**. The cumulative percentage of a 24-hour period that the free drug concentration exceeds the MIC at steady-state pharmacokinetic conditions.

- **In vitro PAE.** The period of suppression of bacterial growth after short exposure of an organism to an antimicrobial compound (unit = time). In this case, drug has been removed.
- **In vivo PAE.** The difference in time for the number of bacteria in a tissue of treated versus control animals to increase $1 \log_{10}$ over values when drug concentrations in serum or at the infection site fall below the MIC (unit = time). The in vivo PAE includes any effect associated with sub-MIC concentrations.
- **Sub MIC effect.** Any effect of an antimicrobial on a micro-organism at concentrations below the MIC (unit = time).
- **Post-antibiotic sub-MIC effect.** The effect of sub-MIC drug concentrations on bacterial growth following serial exposure to drug concentrations exceeding the MIC (unit = time).

These definitions are not without controversy. For example, basing the AUC/MIC or T>MIC upon a 24-hour dosing interval does not address the kinds of alternative dosing intervals approved for use in veterinary medicine. In the US and EU, we see innovative dosing regimens both for fluoroquinolones (e.g., danofloxacin: two doses, 48 hours apart for the treatment of bovine respiratory disease [BRD]) and beta-lactams (e.g., ceftiofur crystalline free acid: one dose for treatment of BRD). Therefore, alternative metrics that are not restricted to traditional daily doses are needed. Furthermore, the assertion that AUC/MIC can be expressed as a dimensionless number is a fundamental challenge. In fact, the units associated with this ratio are hours. By dividing this value by the dosing interval (e.g., 24 hour), we obtain the average plasma drug concentration over the steady-state 24-hour dosing interval relative to MIC, which may be far more informative than the traditional method for expressing this value.

The PD parameter providing the most appropriate surrogate for drug effectiveness is dependent upon several factors. This includes the drug's mechanism of action, whether its effects are time- or concentration-dependent, and the duration of its PAE. A summary of these considerations is provided in Table 5.4. It should be noted that even within a drug class, there may be differences that influence the appropriate PD parameter. Examples of the range of targets proposed for outcome parameters, across a variety of compounds and

targeted pathogens, have been reviewed by Gunderson et al. (2001).

Whether a drug exhibits concentration-dependent or time-dependent killing is largely a function of the shape of its concentration-effect curve. The steeper the curve, the less will be the impact of increasing drug concentrations on the antimicrobial response. Conversely, the more shallow the curve, the greater the relationship between the rate of bacterial kill versus the antimicrobial drug concentration. This relationship can be described using a sigmoidal Emax model, also known as the Hill model, which can be described as follows (Toutain 2002):

$$E(t) = E_0 + \frac{E_{\max} \times C^h(t)}{EC_{50}^h + C^h(t)}$$

where:

$E(t)$ is the effect observed for a given concentration at time t ($C(t)$);

E_{\max} is the maximal effect attributable to the drug; EC_{50} is the plasma concentration producing 50% of E_{\max} ;

h is the Hill coefficient, which adjusts the degree of sigmoidicity in the curve; and

E_0 describes the rate of spontaneous cure.

When $h = 1$, the Hill model reduces to the E_{\max} model, which corresponds to a hyperbolic function.

With the fluoroquinolones, excessively high drug concentrations may result in the inhibition of both RNA synthesis and protein synthesis (Lode et al., 1998). This is also known as the "Eagle Phenomenon".

Developing PK/PD Targets

There are two kinds of PK/PD targets that need to be considered when evaluating antimicrobial compounds: the PK/PD targets associated with pathogen killing and eradication and the PK/PD targets associated with the desired therapeutic response. These two targets may or may not be identical.

The development of clinically relevant PK/PD relationships is no small task, often requiring information on hundreds of patients (Ambrose et al., 2004; Preston et al., 1998). The availability of these large datasets is rare in veterinary medicine. Therefore, alternative sources of information are frequently used to generate initial PK/PD estimates (e.g., in vitro data, animal

Table 5.4. Relationships among drug, drug effects, and the PD surrogate most closely aligned to its clinical response.

Drug	Mechanism	Activity	Bacterial Effect	Duration PAE	PD Parameter
Macrolides Erythromycin, etc.	Binds to 50s ribosome (inhibits protein synthesis).	Static	Time-dependent	Brief*	T>MIC
Azalide				Prolonged	AUC ₂₄ /MIC
Lincosamides (clindamycin)				Brief	AUC ₂₄ /MIC
Ketolides (Telithromycin)				Prolonged	AUC ₂₄ /MIC
Beta-Lactams Penicillins Carbapenems Cephalosporins Monobactams	Inhibits cell wall synthesis.	Cidal	Time-dependent	Gram-negative bacteria: none or brief. Gram-positive bacteria: may be prolonged.	T>MIC
Glycopeptides (Vancomycin)	Inhibit cell wall synthesis.	Cidal (slower than beta lactams)	Time-dependent	Prolonged	AUC ₂₄ /MIC
Fluoroquinolones	Inhibit DNA gyrase, prevents transcription and replication.	Cidal (concentration dependent)	Concentration-dependent	Prolonged	AUC ₂₄ /MIC C _{max} /MIC
Aminoglycosides Gentamicin Tobramycin	Binds to 30s ribosome (inhibits protein synthesis) and disrupts biofilms.	Primarily cidal	Concentration-dependent	Prolonged	AUC ₂₄ /MIC C _{max} /MIC
Tetracyclines Traditional (e.g., Chlortetracycline)	Inhibits protein synthesis at ribosomal level.	Static	Time-dependent	Prolonged	AUC ₂₄ /MIC
Atypical (e.g., Chelocardin and Anhydrochlortetracycline)	Lethal interaction with cytoplasmic membrane.	Cidal	Time-dependent	Prolonged	AUC ₂₄ /MIC
Trimethoprim	Inhibits folic acid synthesis by inhibiting dihydrofolate reductase.	Static alone.	Time-dependent	Brief	T>MIC
Sulfonamides	PABA analogue interferes with folic acid synthesis.	Cidal with sulfonamides. Static	Time-dependent	Brief	T>MIC
Oxazolidinones (Linezolid)	Inhibits initiation of protein synthesis (at 50s ribosomal subunit).	Static (<i>Staph</i> and enterococci) Cidal (most <i>Strep</i>)	Time-dependent	Brief	T>MIC

*Brief: less than one hour. Prolonged: up to six hours.

model studies, and *a priori* information on the activities of a given drug class in relation to the targeted pathogen).

Optimally, PK/PD benchmarks are established for each disease condition by examining a variety of doses and dosing schedules to determine not only the relationship between dose and effect but also to determine if the killing effect reflects a time or a concentration dependency. Alternatively, in products intended for use in food-producing animals, where it may be cost-prohibitive to run extensive studies needed to define the PK/PD relationship, the use of *in vitro* studies (e.g., Lister, 2001; Liu et al., 2005; Zhanel et al., 2001), models of infection in laboratory animals (e.g., Andes

and Craig, 2002; Brouillette et al., 2004), and *ex vivo* studies (Aliabadi et al., 2003) may provide valuable information. The opposite end of the spectrum is the use of a single dose and dosage regimen for demonstrating efficacy. This situation is not appropriate for the rational selection of a given PK/PD index but does provide blood level targets for bioequivalence determinations: we know only that similar effects will be achieved with similar PK profiles, but we cannot determine if drug effectiveness would be improved or reduced with alternative dosage regimens.

While there are certain characteristics common to all antimicrobials within a given drug class, there can be important differences in the PK/PD ratios needed

to achieve a desired effect. Within the fluoroquinolones, it has been demonstrated that the rate of kill (Finberg et al., 2004) and the duration of the in vitro PAE (Finberg et al., 2004; Firsov et al., 1998b) can be markedly different across compounds and microbial species. In some cases, the PK/PD relationship necessary to achieve a 2-log kill can also vary as a function of the microbial strain (Lister and Sanders, 2001; Andes and Craig, 2002). Similarly, the AUC/MIC ratio of 100-125 frequently quoted as a target for fluoroquinolone antimicrobial activity may be an appropriate predictor of success for many Gram-negative infections, but substantially lower AUC/MIC ratios (e.g., 35 to 40) may be appropriate for infections due to Gram-positive organisms (Wright et al., 2000). With regard to the beta-lactams, while there tends to be a substantial in vivo PAE for *Staphylococcus aureus*, a substantially shorter PAE is associated with Gram-negative organisms and streptococcal strains (Craig, 1993).

Veterinarians often deal with a wide range of physiological states and variables such as age, concomitant diseases, or use of concomitant medications. These can influence both drug PK and the responsiveness to therapeutic intervention. The clinical endpoint associated with the use of antimicrobial compounds in companion animal species versus food animal species may not be the same. In companion animals, treatment is aimed at the individual. Conversely, when dealing with food-producing animals, the target is the group rather than the individual and therapeutic objectives may include prophylaxis, metaphylaxis, and/or curative strategies. For some species, the oral route is the only possible modality of administration for mass treatment. Therefore, the use of medicated feeds may only be appropriate for prophylaxis or metaphylaxis, since animals with serious infections tend to be anorexic. When the goal of therapy is maintenance of feed efficiency and minimization of the risk of an infection outbreak, the PK/PD target will differ from the targets associated with a treatment claim.

Within any given bacterial population, there is the possibility of bacterial subpopulations that are less susceptible to the antimicrobial agent. As demonstrated by Blaser et al. (1987) and Drusano (2004), unless these less susceptible pathogens are killed, succeeding microbial generations will re-populate the infection site with pathogens whose MIC values are higher than those found within the initial infection. Accordingly, ensuring adequate exposure following an

initial dose of a fluoroquinolone is as important as insuring that high drug concentrations occur after repeated administration. Drug concentrations need to be adequate to either destroy the existing bacterial population at the site of the infection or (at least) to reduce its size to the point where the host's defense mechanisms can successfully control and eliminate the remaining pathogens.

For drugs exhibiting concentration-dependent killing, C_{\max}/MIC ratios may be particularly important when the pathogen has a high MIC value or is rapidly proliferating (Craig and Dalhoff, 1998). With the latter, rapidly proliferating bacteria have a greater likelihood of undergoing a mutational event that could lead to the genesis of a less susceptible population. In infectious disease processes where there is a high bacterial burden (inoculum effect), the risk of a mutational event is increased due simply to the laws of probability (Craig and Dalhoff, 1998; Drusano et al., 1993). In these cases, to ensure maximum killing, the targeted C_{\max}/MIC ratios are approximately 10 to 12 (Drusano et al., 1993; Preston et al., 1998). Such ratios ensure increased killing of susceptible organisms and an increased killing or inhibition of organisms with higher MICs. The goal in these situations is to reduce bacterial numbers to a level where the host can effectively handle those pathogens not killed by the antimicrobial agent (and to avoid the double-step mutation).

While a high C_{\max}/MIC ratio (e.g., 10) is correlated with a high rate of bacterial kill for compounds exhibiting concentration-dependent killing, there are conditions under which AUC/MIC may be as predictive or more predictive of a sustained antimicrobial activity (Zelenitsy et al., 2003). AUC/MIC also serves as the pivotal PK/PD parameter when the infection is caused by relatively slow growing bacteria, when there is little or no PAE that will contribute to inhibition of bacterial re-growth, or when the MIC for the pathogen is relatively low.

Despite the large body of information suggesting that fluoroquinolones are highly effective in the presence of high C_{\max}/MIC values, exceptions to this rule have been observed. For example, in the case of *Bacillus anthracis*, hollow fiber studies suggest that AUC/MIC was more predictive of success than was C_{\max}/MIC (Deziel et al., 2005). This result relates to the findings described by MacGowen et al. (2001a, 2001b), where time to kill 99% of the inoculum depends on C_{\max}/MIC , but the ability to maintain this

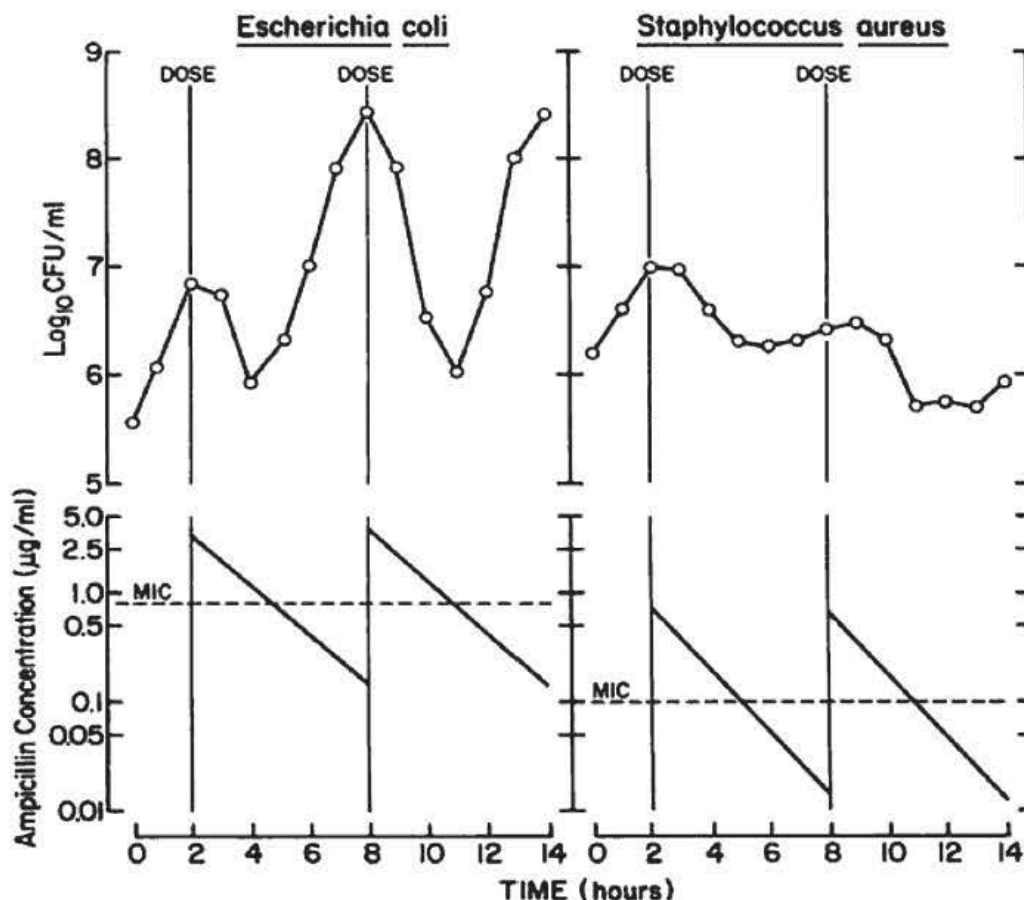


Figure 5.5. Effect of two simulated intravenous bolus injections of ampicillin on the CFU/mL of *E. coli* ATCC strain 12407 and *S. aureus* ATCC strain 25923 in an in vitro dialysis kinetic model. From Toothaker, et al. (1982) with permission.

decrease in microbial counts is related to AUC/MIC (in vitro test conditions). If the duration of time between doses is extended beyond 24 hours, effectiveness may also depend upon $T > \text{MIC}$ (MacGowan et al., 2001a).

Post-Antibiotic Effects (PAE)

High drug concentrations relative to the MIC may contribute to an increase in the duration of the in vitro and in vivo PAE. For those bacteria/drug combinations that exhibit a PAE, in vivo PAEs have been shown to be longer than in vitro PAEs for most organisms. Beta-haemolytic streptococci are notable exceptions. Thus, optimizing the C_{max} -to-MIC ratio will delay the re-growth of the pathogen, sometimes by several hours. This type of dosing regimen results in fewer organisms remaining that can evolve into a resistant sub-population.

For many compounds, the duration of the in vivo

and in vitro PAE is substantially greater for Gram-positive than for Gram-negative pathogens. An example of this is seen in Figure 5.5, where the in vitro PAE of ampicillin is compared in Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) species. Because the duration of the in vitro and in vivo PAE of beta-lactams tends to be negligible for Gram-negative species, it is often recommended that concentrations of drug remain above the MIC of the pathogen for $> 80\%$ of the dosing interval. In contrast, a $T > \text{MIC}$ of about 40% is considered to be adequate for staphylococcal species.

This difference in the duration of the in vitro and in vivo PAE may also be one of the reasons why the in vivo AUC/MIC for fluoroquinolones tends to be less for Gram-positive than for Gram-negative organisms. For Gram-negative organisms, the estimated AUC/MIC ratios needed to ensure effective treatment and prevent the selection of resistant strains is estimated to be approximately 100 to 125 (Forrest et al., 1993). In

contrast, the AUC/MIC ratio for Gram-positive bacteria is considerably lower, approximately 30 to 50 for a number of drug/microbe combinations (Ibrahim et al., 2002; Preston et al., 1998; Wright et al., 2000). Studies involving the third and fourth generation fluoroquinolones suggest that for Gram-positive organisms AUC/MIC values are substantially lower when C_{\max} /MIC values are ≥ 10 (Nightingale et al., 2000).

Recently developed mathematical models help predict the duration of the in vivo PAE. Mouton and Vinks (2005a, 2005b) described the duration of in vivo PAE as a function of several variables. These include: (1) the terminal elimination half-life ($T_{1/2}$) of the drug in the dosage form; (2) the estimated rate of bacterial killing (ϵ); (3) the slope of the curve relating the magnitude of bacterial kill versus drug concentration (γ , the Hill Coefficient); (4) the estimated number of viable bacteria at the infection site, the maximum number of bacteria (or attainable bacterial density), and the concentration associated with 50% of the maximal effect (EC_{50}); and (5) the MIC of the targeted pathogen.

Considerations associated with the development of a concentration-versus-effect model, from which terms such as E_{\max} , EC_{50} and γ can be derived, are reviewed elsewhere (Toutain, 2002; Toutain et al., 2002).

Using the relationship between these parameters and the MIC of the targeted pathogen, the drug concentration associated with zero change in bacterial numbers (also called the stationary concentration, SC) can be estimated. This is then used to estimate the duration of antimicrobial activity associated with a specific dose and, therefore, can be used as a tool for selecting the dosing interval. In general, the in vivo SC tends to exceed the in vitro SC because the in vivo bacterial growth rate (λ) tends to be slower than that observed in vitro. Therefore, this mathematical approximation method will generally provide a conservative approach for estimating an appropriate dosing interval.

The Inoculum Effect

Retrospective work on doxycycline in swine indicates that although PK/PD strongly suggests that at least a 20 mg/kg/day dose is necessary to insure clinical success in the treatment of swine respiratory disease, a dosage of 11 mg/kg/day in feed effectively controls swine pneumonia due to *Pasteurella multocida* (Toutain, 2005; Bousquet et al., 1998). This disparity may,

at least in part, be related to an inoculum effect, where differences in PK/PD targets may be attributable to the relatively small number of colony forming units (CFU) in these animals treated according to a metaphylactic strategy (i.e. before establishment of a full disease and expansion of the inoculum). There may also be important non-antibiotic effects associated with the use of doxycycline (e.g., its anti-inflammatory activity or its effect on pathogen attachment to host tissues) that cannot be explored using in vitro test procedures.

Numerous studies have examined the influence of inoculum size on the killing activity of antimicrobial compounds, with the claim that the size of the inoculum influences the MIC value and the amount of drug needed to obtain a 3-log kill (a bactericidal effect). In some cases, this observation is artificial, reflecting in vitro test conditions and the effect of confined volume on the relationship between bacterial concentration and the concentration of bacterial-generated hydrolyzing enzymes (Craig et al., 2004). For this reason, Craig et al. concluded that the inoculum effect has little clinical consequence. On the other hand, in vivo inoculum effects have been observed, affecting levofloxacin static concentrations and, to an even greater extent, the bactericidal AUC/MIC ratios, a finding postulated to result from a microbial population burden that exceeds the mutation frequency (e.g., Jumbe et al., 2003). This is illustrated in Figure 5.6. Under conditions of high microbial burden, this inoculum effect has also been attributed to a decrease in oxygen tension (Morrissey and George, 1999). Similarly, markedly higher doses of several carbapenems and fluoroquinolones (expressed as ED_{50}) were needed for the treatment of mice infected with *S. aureus* and *P. aeruginosa* infections (Mizunaga et al., 2005).

The relationship between bacterial number and therapeutic effect may be of particular clinical significance in situations where there is purulent material because bacterial concentrations may be as high as 10^8 to 10^9 CFU/mL (König et al., 1998).

Shortcomings in the Use of in vitro Data to Determine the PK/PD Target

Blood concentrations and MIC data alone cannot predict drug effectiveness. For example, using human and bovine estimated breakpoints for cephapirin and oxytetracycline (the latter not approved for use in the treatment of mastitis), Constable and Morin (2002)

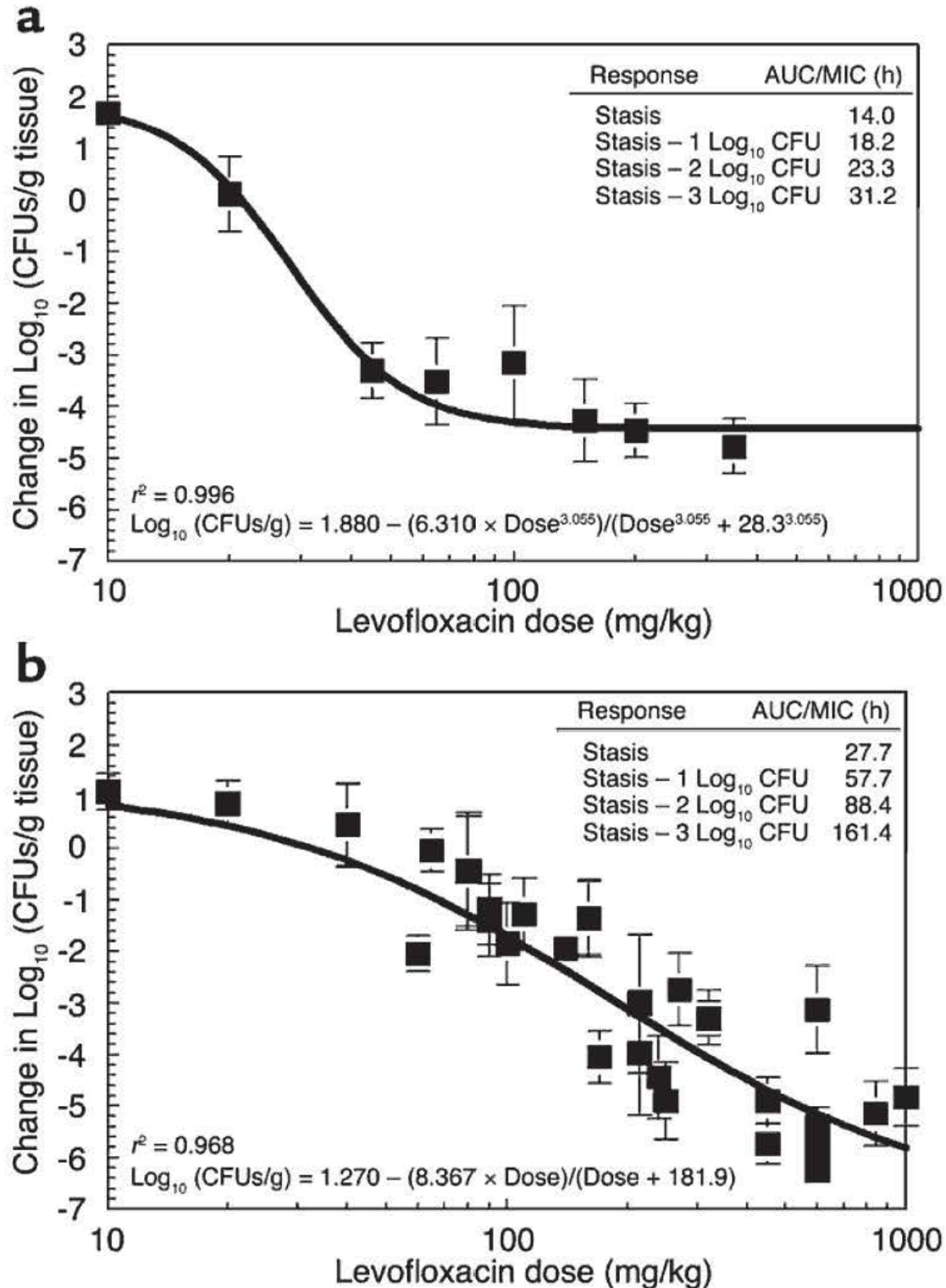


Figure 5.6. *P. aeruginosa* dose response. Normal mice were inoculated with about 10^7 (a) or 10^8 (b) bacteria per thigh. The levofloxacin MIC and MBC were 0.8 $\mu\text{g/mL}$ and 1.6 $\mu\text{g/mL}$, respectively. The x-axis displays the exposures in mg/kg doses. The model allowed calculation of the dose necessary to achieve stasis (i.e., to return the colony counts at sacrifice to that used for the challenge), as well as 1, 2, and 3 log_{10} (CFUs/g) reductions in bacterial counts from the stasis point. From Jumbe et al. (2003) with permission.

showed that the MIC values predicted that the causative pathogens would be susceptible to both agents. However, these compounds were not effective in the treatment of acute bovine mastitis. Similarly, compounds effective in the treatment of acute bovine mastitis may be ineffective in the treatment of chronic bovine mastitis (Owens et al., 1997).

Drug potency is often considered in terms of MIC, which is a measure of a drug's static effect on microbial growth. The MIC may not be the same as a compound's minimum bactericidal concentration (MBC). Since both the MIC and MBC values are in vitro estimates, they do not reflect a drug's rate of killing, the effect of serum on antimicrobial activity, PAE, or post-antimicrobial sub-MIC effects (Craig and Dalhoff, 1998). Therefore, the clinical relevance of MIC values has been called into question by numerous investigators (Mueller et al., 2004; Firsov et al., 1998a, 1999). Firsov et al. (1999) demonstrated that even with comparable AUC/MIC ratios, different fluoroquinolones can have markedly different in vitro time-kill profiles. Such differences can not only influence the selection of an AUC/MIC target value (and therefore dose and dosing frequency), but also the potential for selecting for resistant bacterial strains.

Traditional in vitro susceptibility data reflect the impact of therapeutic agents on bacteria that are in the active growth phase associated with free-floating (planktonic) cells. These tests do not describe the differential activity across the various life phases of the bacteria (Cerca et al., 2005). As bacteria form biofilms, thereby entering a non-growth phase, many compounds begin to lose their antimicrobial effects. This very important distinction cannot be predicted when MIC alone is used as the PD component of the PK/PD relationship, again underscoring the importance of basing PK/PD assessments on clinical effectiveness data.

MIC values cannot distinguish between cidal and static effects: a critical point for cidal compounds. Therefore, several endpoints, such as the time to kill 99% of the initial inoculum, log change at 24 hours, and kill curves, have been proposed as alternative in vitro measures for assessing antimicrobial effects at specific drug concentrations within the biophase (MacGowan and Bowker, 2002). However, these alternative measures have not yet been adequately standardized, leading to difficulty with inter-study interpretation. Nevertheless, this information, when combined with MIC values and in vivo effectiveness (even if based

only on animal model data), can provide a relatively inexpensive tool for developing PK/PD targets.

There are numerous examples of traditional susceptibility tests failing to adequately reflect in vivo drug activity. For example, beta-lactams are inactivated by purulent material due to the accumulation of bacterial beta-lactamases; gentamicin can be inactivated by reversibly binding to DNA released from lysed neutrophils; netilmicin and amikacin are inactivated by disrupted leukocytes (Labro, 2000). Owens et al. (1997) noted that the bacteriologic cure rate for newly acquired *S. aureus* intramammary infections (IMI), which they defined as infections of less than two weeks duration, was 70% when treated with a penicillin and novobiocin combination. However, the cure rate dropped to less than 35% for chronic infections (those lasting longer than four weeks). Accordingly, they observed that while the successful treatment of acute infections could be predicted on the basis of in vitro susceptibility test results, this was not the case for chronic *S. aureus* infections. It is not known if this outcome is due to the driving of *S. aureus* infections from extracellular to intracellular sites (Brouillette et al., 2004), to biofilm formation (Cucarella et al., 2004), or to some other bacterial pathogen-host interaction is unknown.

In addition, there are actions of antimicrobial compounds other than their direct microbial cidal or static actions that can influence their therapeutic effectiveness. These factors also need to be considered when targeting a dosage regimen. This emphasizes the importance of clinical trials to validate predictions generated on the basis of preclinical and in vitro study data.

Possibly the most important shortcoming of traditional in vitro susceptibility test methods is that they cannot describe the impact of a drug on a pathogen's virulence factors. These factors are responsible for anchoring to and invading host cells, quorum sensing (bacterial production of autoinducers that serve to regulate gene expression within the bacterial colony), and the production of toxins and factors that influence host immune functions (Alksene and Projan, 2000). The propensity to form biofilms is considered one of the major virulence factors involved in coagulase-negative staphylococcal infections (Otto, 2004). Drugs that act on any of these virulence factors may be far more effective than would be predicted solely from static effects on planktonic organisms. Therefore, PK/PD relationships for agents that act upon these

virulence factors cannot be based solely upon traditional PK-MIC considerations.

Bacteriostatic versus Bactericidal: Is There a Preference?

While drugs are often categorized as bactericidal or bacteriostatic, there are numerous instances where compounds can exhibit both kinds of effects. For example, at concentrations close to the MIC of a given pathogen, fluoroquinolones act as bacteriostatic rather than bactericidal agents. At clinically relevant concentrations, chloramphenicol, which is bacteriostatic against most Gram-negative bacteria, is cidal against *Haemophilus influenzae* and *Streptococcus pneumoniae* (Feder, 1986). Similarly, linezolid is bacteriostatic against enterococci and staphylococci, but is bactericidal for most (not all) streptococcal strains. The bactericidal activity of a drug can vary with intracellular pH, oxygen content, and intracellular enzymatic activity (Butts, 1994). Therefore, it is necessary to consider each drug-microbe combination independently to accurately characterize the rate and nature of a drug's effect.

When it is preferable to administer a bacteriostatic versus a bactericidal agent? The answer depends upon the host immune response and the type of disease process. Certain antimicrobial agents such as beta-lactams may enhance the rate of endotoxin release through slow kill mechanisms, thereby resulting in higher patient mortality (Morrison, 1998). This was observed when comparing the effect of certain antimicrobials on endotoxin release and tumor necrosis factor α (TNF- α) production in an in vitro model of septicemia in foals (Bentley et al., 2002). In this regard, bacteriostatic compounds often result in a lower release of endotoxins as compared to many cidal drugs such as the beta-lactams and, to some extent, the fluoroquinolones (Prins et al., 1994). When a primary cause of host pathology is the release of bacterial endotoxins, the capability of the antimicrobial to inhibit bacterial protein synthesis may be more important than its static versus cidal activity (Bottcher et al., 2004). Considering the significant clinical effect of endotoxins, there is currently an effort to identify antimicrobials that can sequester and neutralize these toxins (Zorko et al., 2005). Among the existing antimicrobials, the aminoglycosides may bind to and possibly neutralize endotoxins (Prins et al., 1994), although the clinical relevance of this finding has recently been questioned (Goscinski et al., 2004).

If cidal drugs are used in situations associated with endotoxemia, kill must be rapid (Uemura et al., 2004). The rapid bactericidal activity of fluoroquinolones can be advantageous in treating chronic airway infections caused by *Pseudomonas aeruginosa*, thereby suppressing excessive immune-mediated responses and preventing the progression of tissue damage (Sato et al., 1997).

Other Factors that Influence the Clinical Response to Drugs

Across the various antimicrobial classes, comparable free serum concentrations and in vitro antimicrobial activity (expressed as a MIC) does not necessarily equate with clinical effectiveness (Stevens et al., 1995). On occasion, drugs that are ineffective in vitro are found to be effective in vivo. Often, this unexpected level of effectiveness is related to interactions among the antimicrobial agent, the host, and the pathogen. To fully appreciate these additional drug effects, we need to consider some of the fundamental aspects of the host defense response. The complex interaction between the host immune system and the invading pathogen has been summarized in a review by Labro (2000) and includes:

- A localized and beneficial inflammatory response is generated by local resident macrophages in response to pathogen entry into the host. This includes the production and release of humoral agents such as cytokines and activated complement. These result in a chemotactic gradient that attract polymorphonuclear leukocyte (PMNs) to the infected area, modify endothelial cell membrane receptors, and promote a slowing of the blood flow. In addition, the coagulation cascade may be triggered and the local lymphatic flow impaired. The benefit to the host derived from this blockade of extracellular fluid drainage is that it impedes microbial invasion to other systemic sites. This explains the adaptive advantage associated with local edema, node enlargement, etc.
- PMNs respond to the chemotactic substances and begin to adhere to endothelial membranes. This results in transendothelial migration (diapedesis), followed by an oriented migration (chemotaxis) toward the inflammatory site.

- Upon arrival at the inflammatory site, the PMNs can recognize pathogens via their membrane receptors for opsonins (e.g., complement factors and components of immunoglobulins) and phagocyte lectins (opsonin-independent phagocytosis). Both opsonin-dependent and independent activation of PMNs trigger phagocytosis.
- The engulfed microbes cause the PMNs to release substances that destroy the invading pathogen in their phagolysosome.
- The PMNs are also involved in intercellular communication that modulates the activity of other immune responses (e.g., monocyte/macrophage function) as well as tissue repair. However, this immune response can have a detrimental effect, resulting in host tissue destruction. For example, neutrophils must defend themselves against the oxidants they produce, and when released to surrounding tissues, these oxidants can result in an environment hostile to host tissues.

There is a growing body of data indicating that antimicrobial compounds can influence these immune functions. An example of this is the enhanced effectiveness of fluoroquinolones against some infection models involving *B. fragilis*, which has been attributed to an enhanced immune response (Dalhoff and Shalift, 2003). Similarly, there are occasions where effectiveness has been associated with subtherapeutic doses of compounds. These effects often depend on alterations in cytokine production or the production of other inflammatory intermediates. For example, some compounds were found to improve non-infectious diseases (Amsden, 2005). Phagocyte alteration of pathogen metabolism or structure may render the pathogen more susceptible to the effect of the antimicrobial agent, and drug concentrations too low to have a cidal effect may render the microbe more susceptible to leukocyte action, an event referred to as post-antibiotic leukocyte enhancement (McDonald et al., 1981).

The multifaceted nature of potential drug-host-pathogen interactions is graphically illustrated in Figure 5.7.

The sections which follow cite specific examples of these interactions (van del Broek, 1989; Labro, 2000).

Phagocytosis and Killing

- Tetracyclines and bacitracin inhibit phagocytosis by binding to divalent cations.

- Sulfonamides and trimethoprim decrease intracellular killing by interfering with H_2O_2 production.
- SubMIC concentrations of clindamycin and lincomycin increase phagocyte uptake of *Streptococcus pyogenes* by affecting bacterial surface proteins.
- Penicillin G is ineffective on *S. aureus* when that bacterium is ingested by PMNs. However, it has significant activity against *S. aureus* when phagocytized by monocytes. It would appear that this enhanced activity relates, at least in part, to monocyte production of substances that are synergistic with penicillin.

Chemotaxis of Granulocytes

- An in vitro chemotaxis is observed when *E. coli* is grown in the presence of either ceftazidime or ampicillin.
- Gentamicin, erythromycin, and minocycline depress chemotactic activity of culture filtrates of *Propionibacterium acnes*.
- Sub-MIC concentrations of tetracyclines decrease chemotaxis by binding to divalent cations.

There are many examples where additional effects, such as inhibition of bacterial toxin production via inhibition of microbial protein synthesis (Shryock et al., 1998) and anti-inflammatory activity (Dalhoff and Shalift, 2003) can have a very important role in clinical outcome. For example, Slocumbe et al. (1985) demonstrated that the tissue damage associated with acute bovine pleuropneumonia caused by *Mannheimia haemolytica* consisted of mildly scattered microscopic lesions in neutropenic calves, whereas in neutrophil-sufficient calves that received the same challenge there was extensive fibrinopurulent inflammation of the pulmonary tissue. The lesions in the neutrophil-sufficient animals consisted of marked interlobular edema, fibrinous exudates, alveolar hemorrhage and endothelial swelling. These findings suggested that bovine neutrophils were important in mediating some of the damage seen in the pulmonary parenchyma following the introduction of *M. haemolytica* (Slocumbe et al., 1985).

For any drug, proinflammatory cytokines, such as interleukin 8 (IL-8), IL-1, IL-6, colony stimulating factor (CSF), and TNF- α mediate the host immune response. These molecules tend to attract macrophages, monocytes and polymorphonuclear neutrophils (PMNs) to the site of the infection. Despite the obvi-

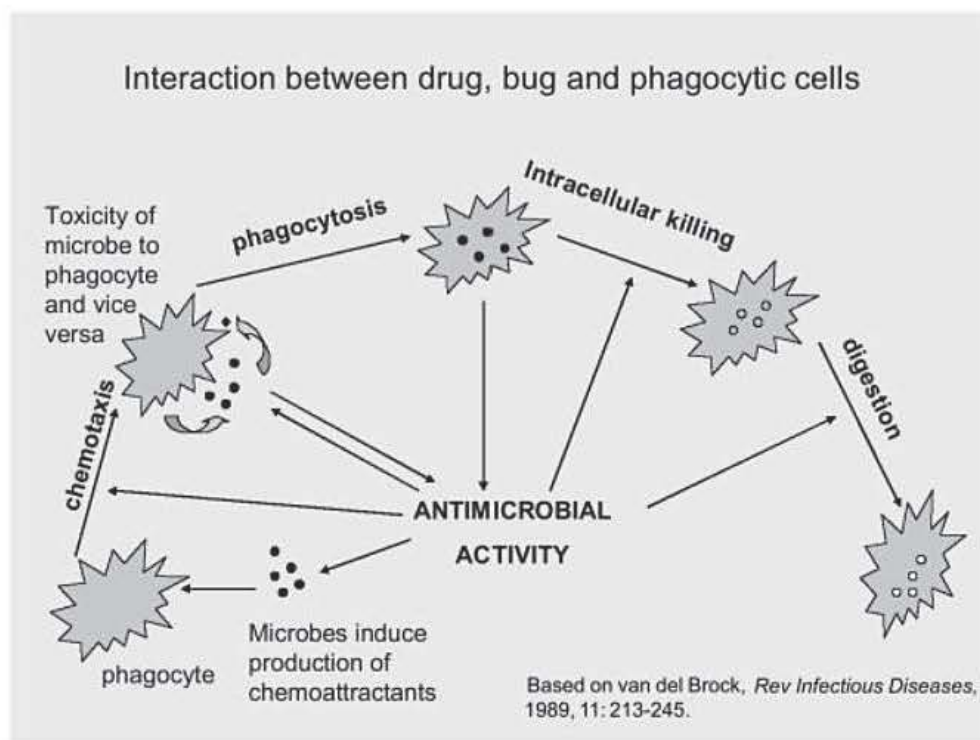


Figure 5.7. Examples of drug effects involved with phagocyte-microbe interaction. Based on van del Brock, et al., 1989.

ous importance of the host immune response in combating acute infections, the excessive synthesis and secretion of inflammatory mediators can lead to morbidity and mortality. In such cases, antimicrobials that can minimize the secretion of these pro-inflammatory cytokines can provide a significant therapeutic benefit (Labro, 2000). Many antimicrobials have been shown to interfere with or reduce the secretion of cytokines, thereby controlling the pro-inflammatory process (Reato et al., 2004). This interference tends to be targeted to specific cytokines. Furthermore, these actions tend to vary across compounds within a class, across the mechanism of immune response stimulation, and with drug concentration (Reato et al., 2004).

These additional influences are excluded from analysis when we set our PK/PD targets solely upon a drug's antimicrobial effects. Particularly in the case of macrolides, the traditional PK/PD paradigm, when primarily based upon in vitro concentration-effect data, may not adequately reflect clinical outcomes. However, such effects can be incorporated into targeted serum concentrations when the PK/PD model is based upon a retrospective analysis of clinical outcome (Sánchez-Navarro et al., 2001; Drusano et al., 2004).

Biofilms

Chronic infections differ from acute infections, the former being associated with biofilm formation and slow cell division rates (Owens et al., 1997). In these situations, in vitro MIC values alone cannot predict in vivo activity of a drug.

The issue of biofilms is very challenging and may be of substantial clinical consequence in numerous veterinary disease conditions (Costerton et al., 1999). Even if active against pathogens during the non-growth phase, a drug still may not be effective in combating chronic infections. For example, fluoroquinolones cannot completely eradicate urinary tract infections (Kumon, 2000). Although the possibility of more complex difference in the complexity of in vivo versus in vitro biofilms has been suggested as a possible reason for this resistance, the ability of some pathogens to form intracellular inclusions should not be overlooked. These inclusions exist in the host tissue, and are released when the tissue is sloughed. Release from the host cell results in re-infection, hence the chronic nature of this condition.

Nearly all bacteria are capable of forming biofilms, and biofilms have been postulated to exist in up to 65% of all human infections (Potera, 1999). There are

several factors that may contribute to antimicrobial failure in the treatment of biofilms, and mathematical models have been developed to describe these multifactorial interactions (Keren et al., 2004a, 2004b; Cerca et al., 2005; Cogan et al., 2005). Variables include:

- Penetration failure: Although some investigators believe this is due to diffusion barriers, it is more likely that components of the biofilm may interact with and neutralize the antimicrobial compound.
- Some antimicrobials require that the pathogen is in an active growth phase. Therefore, these drugs will be ineffective against bacteria that are in a quiescent phase. However, some investigators conclude that even with prolonged drug exposure, biofilms tend to retain a population of “persister cells” that remain unaffected by an antibacterial challenge. These persister cells remain dormant and are thus unaffected by the cidal effects of antimicrobial agents, even those that are active against slow-growing cells.
- Quorum-sensing has been proposed as a mechanism by which bacteria can up-regulate resistance mechanisms. Recent studies suggest that interference with the quorum-sensing communication system may increase bacterial susceptibility.
- Some investigators suggest the emergence of biofilm-specific phenotypes. Much of the work done on bovine mastitis has supported this postulate.

Each of these aspects of biofilm physiology exerts an effect on antimicrobial activity that is drug-specific (König et al., 2001).

Some investigators are now promoting the use of antibiotics by pulse dosing for conditions in which biofilms likely play a role (e.g., cephalosporins for dog dermatitis). For example, they may recommend treating dogs systemically for three days per week for the entire life of the animal. Considering what is now known about biofilms, such pulse dosing will only periodically control the release of planktonic pathogens but will not eradicate the biofilm.

Drug Use in a Population

The use of “mean” PK estimates (e.g., average AUC_{0-24} value or average $T > MIC$) does not take into account the uncertainties that influence the range of responses

to a compound when used in a real-life patient population. As stated by Ambrose and Quintiliani (2000), “It is important to remember that population pharmacokinetic and microbiological data are stochastic in nature and analytically need to be treated as such.” For this reason, Monte Carlo methods provide an excellent mechanism for examining the probabilistic outcomes within a range of MIC values (Drusano et al., 2001; Drusano, 2004).

While individual data obtained under laboratory conditions provide important information on the pharmacokinetics of the drug, we need to consider whether or not these data truly reflect the kinetics of the drug under field conditions. For example, changes in drug clearance due to compromised hepatic or renal function can result in higher-than-anticipated drug concentrations, which could be a positive outcome if the drug is safe. On the other hand, the availability of active drug concentrations at the site of infection may be compromised. This is particularly problematic when, due to either sepsis or swelling at the infection site, there is a decrease in the delivery of drug from the systemic circulation. Additional reasons for differences between tissue concentrations in normal versus healthy individuals include such factors as changes in drug diffusivity through the infected tissues and changes in concentration of nonionized drug due to the relationship between drug pKa and the pH at the site of the infection.

There can be significant changes in pharmacokinetics as a function of breed, age, gender, and species. For example, using population methods, Preston et al. (1998) noted that substantially higher levofloxacin AUC_{24} values were needed to achieve therapeutic success in older as compared to younger human patients. Their contention for this finding related to the physiological status of the patient. For this reason, when assessing the likelihood for success within a population of potential recipients, population variability in PK, as well as variability in MIC values, needs to be considered. Differences in drug metabolism can also occur depending upon whether or not an animal is castrated (Skálová et al., 2003).

Monte Carlo simulation procedures are often used for generating population predictions. Experimentally generated estimates of parameter means, variances and relevant covariate information (e.g., age, gender, breed, creatinine clearance) are used to generate PK parameter distributions that conform to their respec-

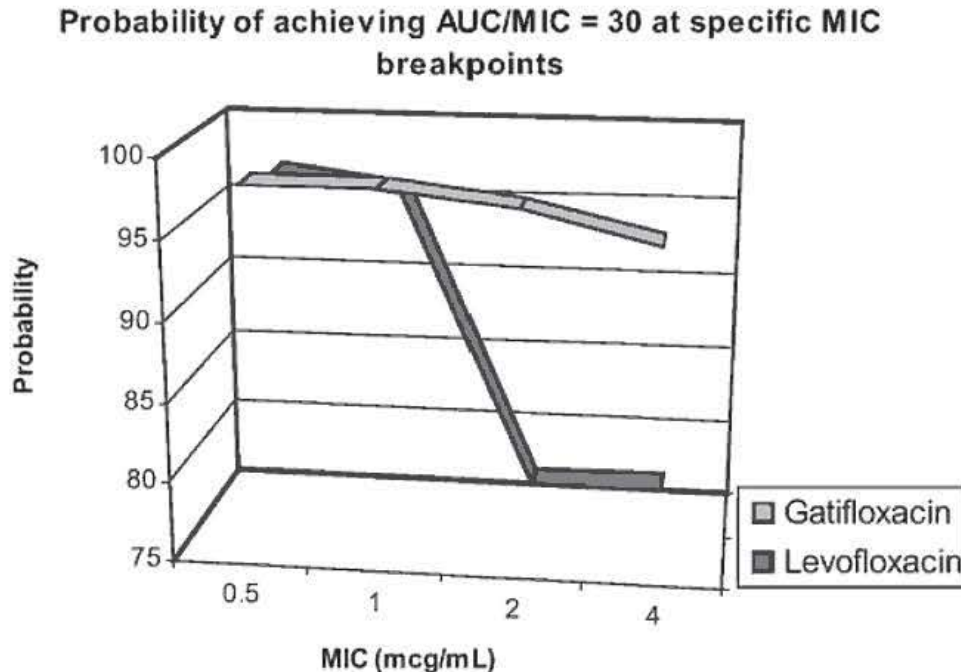


Figure 5.8. Probability of achieving an AUC:MIC ratio of 30 at specific MIC breakpoints. Based on Ambrose and Grasela, 2000.

tive probabilities. From these randomly generated values, the simulation procedure generates a large number (generally thousands) of PK/PD values that can be used to assess the probability of achieving specific PK/PD values for any specified MIC. This simulation outcome is not weighted by the probability of achieving a specific MIC value but rather examines the likelihood of achieving some PK/PD target for any MIC value in question. Figure 5.8 provides an example of the use of MICs to compare the likelihood of achieving an AUC/MIC value of 30 with the approved doses of gatifloxacin and levofloxacin (Ambrose and Grasela, 2000). The value of 30 was determined on the basis of human survival data.

An underlying assumption in the use of each of these methods is that we have a true representation of the MIC distribution in the bacterial population. Likewise, we assume that the PK estimates adequately represent the patient population.

There are several ways to generate these simulations (Dudley and Ambrose, 2000). The pivotal PK metric based upon a population estimate of mean and variance for that parameter can be simulated. For the microbial susceptibility, the proportion of the patient population expected to reach the targeted PK/PD relationship (e.g., AUC/MIC = 30) can be examined.

The probability of obtaining that targeted PK/PD value using fixed MIC values (e.g., Figure 5.8) is then determined.

When the targeted PK/PD parameter is time above MIC ($T > MIC$) or the ratio of peak concentrations to MIC (C_{max}/MIC), a more scientifically robust approach would be to simulate the PK profiles based upon mean vectors and variance/covariance matrices of the various pharmacokinetic parameters (e.g., volume of distribution, clearance, percent absorbed). Population PK parameter values from these simulated profiles are obtained and then the target attainment based upon the use of fixed MIC values is estimated.

Optimally, one would simulate the PK/PD parameter distributions based upon simulations that factor both the distribution of the PK parameters (using either of the two approaches described above) and the MIC population distribution. Using this approach, each simulated individual is randomly assigned an MIC value based upon probability distribution derived from the antibiogram.

Another approach is to examine the percent of the population that will reach a specified PK/PD target as dose is varied. With this estimation procedure, the PK/PD population distribution obtained by MICs is weighted by the population distribution of MIC values

associated with the pathogen. In this case, the probability of achieving the PK/PD target for a given MIC value is multiplied by the percentage of the microbial population associated with that MIC value. This is considered to be a weighted Monte Carlo Simulation. When the weighted probabilities are summed across all of the MIC values, we obtain an overall weighted target attainment rate (weighted TAR) for that dose. By repeating this across doses, we can then determine which dose is consistent with the magnitude of risk we are willing to tolerate. An example of the determination of the weighted target attainment, similar to the one published by Drusano et al. (2001), is provided in Table 5.5.

When conducting Monte Carlo simulations, the resulting weighted TAR is directly related to the desired magnitude of the killing effect. For example, a much lower target (and consequently, lower dose) may be appropriate for effectively treating a population if the microbiological goal is stasis. However, very high concentrations may be needed to insure therapeutic success if rapid and extensive killing is necessary. Andes and Craig (2002) observed that to achieve stasis, 1-log kill and 2-log kill for *S. aureus* infection in the thighs of neutropenic mice, AUC/MIC ratios of 69.7, 129 and 235, respectively, were needed. Similarly, ratios to achieve the same goal for this thigh infection model in non-neutropenic mice were 32.2, 62.2 and 165, respectively. The level of kill needed to treat any infection is a subjective question for which patient response, potential risk of antimicrobial drug resistance, cost and safety (both for the target animal species and human food safety) must be weighed. This is a judgment call which cannot be definitively solved by any mathematical technique.

Consequence of Population PK and PD Characteristics on the Weighted Target Attainment Rate

It is important to recognize that due to the population distribution of drug PK and the distribution of pathogen MIC values, it is incorrect to assume that a doubling of the dose will necessarily result in doubling of the weighted target attainment rate. As seen in the simulated example provided in Figure 5.9, the benefit of increasing the dose depends largely upon the shape of the dose/weighted TAR curve. When nearing a plateau, a doubling of the dose will have minimal ad-

Table 5.5. Estimation of a weighted target attainment rate.

MIC	%AUC/MIC=100	% bacteria w/MIC	Product of fraction
0.125	0.99	0	0
0.25	0.94	0.3	0.282
0.5	0.57	0.35	0.1995
1	0.09	0.2	0.018
2	0.03	0.1	0.003
4	0	0.05	0
			Sum=0.5025

ditional therapeutic benefits but will likely result in substantial deleterious effects associated with target animal safety, human food safety, and cost. On the other hand, when the dose is on the linear portion of the profile, substantial benefit may be achieved by increasing the dose or the frequency of administration. Therefore, this kind of analysis would be of enormous benefit during attempts at dose optimization.

Conclusion

What is the role for traditional PK/PD metrics in antimicrobial therapy? The answer to this question is clear: PK/PD provides the basis for selecting a starting point for dose prediction. If concentrations are suboptimal, the use of the antimicrobial may increase the risk of driving an infection into chronic form or selecting for resistant pathogenic strains. In this regard, animal model studies and in vitro investigations provide valuable insights into the exposure-response relationship for the planktonic forms of the pathogen. We need to keep in mind that an antimicrobial agent can produce a short-term therapeutic success but may contribute to long-term therapeutic failure. Bovine mastitis and urinary tract infections are two excellent examples in which this phenomenon occurs. Particularly for those classes of compounds where concentration-response relationships have been well defined, PK/PD can help avoid the selection of doses that can lead to therapeutic failures in both the long and short terms.

There are numerous examples in which antimicrobial agents expected to be highly effective failed to produce the desired clinical outcome. Reasons for this may include the growth phase of the bacteria, release of toxins, and in vivo drug inactivation. There are also

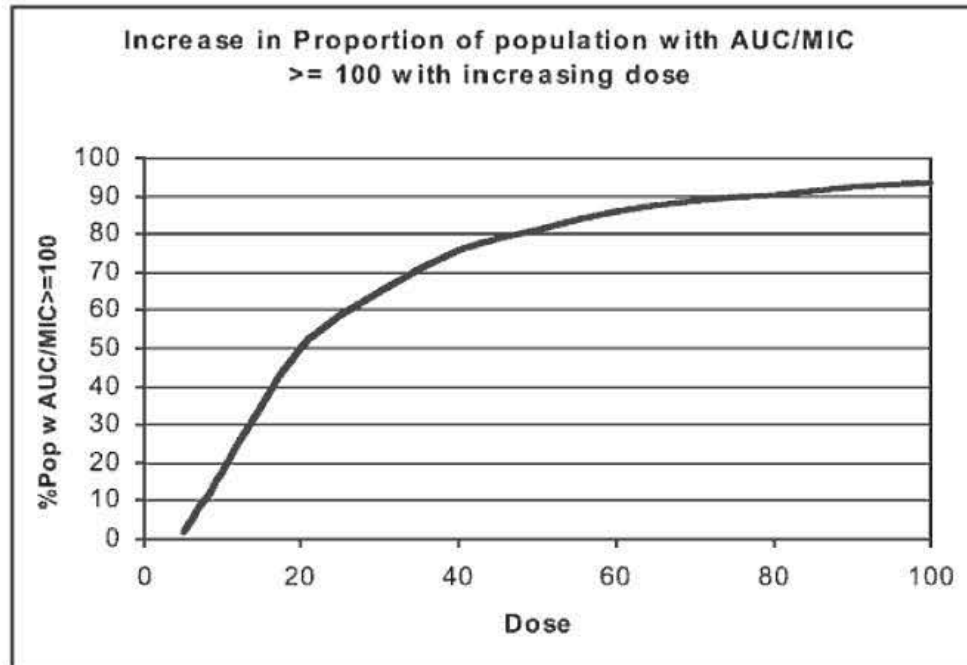


Figure 5.9. Impact of increasing the dose on the percent of the simulated population achieving the PK/PD target of $AUC/MIC \geq 100$.

examples of clinical cures at doses that were not expected to be effective. Reasons for this outcome may include drug effects on toxin production, the enhancement of host immune responses, and drug anti-inflammatory properties. These observations all point to the fact that antimicrobial agents do far more than simply inhibit or kill bacterial pathogens. These are complex compounds that precipitate an array of events, any of which may produce a therapeutic or adverse response. Attempts to summarize such complexities as a simple two-dimensional AUC/MIC , C_{max}/MIC , or $Time > MIC$ metric inherently assume that it is only the killing (inhibition) of the planktonic cell that is of therapeutic relevance. This clearly is an incorrect assumption. Optimally, concentration-effect controlled clinical trials help to establish whether a predicted dose is appropriate. When such data are available, the appropriate PK/PD relationship (ratios) for dose optimization can be defined. In addition to short-term effectiveness, long-term maintenance of an effective therapeutic arsenal must also be considered.

The scientific community needs to strive to understand the mechanisms of action for each new molecular entity, because it is only through this understanding that we can truly define the PK/PD relationships for these compounds and the host factors that affect

the response to therapy. Ultimately, because of the numerous complex interactions that can influence a drug's effect, it is only after years of actual field experience that we can have a higher level of certainty that a drug will be safe, effective, and produce minimal risk of long-term therapeutic failures when administered to the targeted patient population.

Bibliography

- Agerso H et al. 1998. Penetration of amoxycillin to the respiratory tract tissues and secretions in *Actinobacillus pleuropneumoniae* infected pigs. *Res Vet Sci* 64:251-257.
- Aliabadi FS et al. 2003. Pharmacokinetics (PK), pharmacodynamics (PD), and PK-PD integration of danofloxacin in sheep biological fluids. *Antimicrob Agents Chemother* 47:626-635.
- Alksene LE, Projan SJ. 2000. Bacterial virulence as a target for antimicrobial chemotherapy. *Curr Opin Biotechnol* 11:625-636.
- Ambrose PG, Grasela DM. 2000. The use of Monte Carlo simulation to examine pharmacodynamic variance of drugs: fluoroquinolone pharmacodynamics against *Streptococcus pneumoniae*. *Diagn Microbiol Inf Dis* 38:151-157.
- Ambrose PG, Quintiliani R. 2000. Limitations of single point pharmacodynamic analysis. *Ped Infect Dis J* 19:769.
- Ambrose PG et al. 2004. Use of pharmacokinetic-pharmacodynamic and Monte Carlo simulation as decision support for the re-evaluation of NCCLS cephem susceptibility breakpoints for enterobacteriaceae. *ICAAC*, Abstract #138.

- Amsden GW. 2005. Anti-inflammatory effects of macrolides—an underappreciated benefit in the treatment of community-acquired respiratory tract infections and chronic inflammatory pulmonary conditions? *J Antimicrob Chemother* 55:10.
- Andes K, Craig WA. 2002. Pharmacodynamics of the new fluoroquinolone gatifloxacin in murine thigh and lung infection models. *Antimicrob Agents Chemother* 46:1665.
- Bentley AP et al. 2002. Antimicrobial-induced endotoxin and cytokine activity in an in vitro model of septicemia in foals. *Am J Vet Res* 63:660.
- Blaser J et al. 1987. Comparative study with enoxacin and netilmicin in a pharmacodynamic model to determine importance of ratio of antibiotic peak concentration to MIC for bacterial activity and emergence of resistance. *Antimicrob Agents Chemother* 31:1054.
- Bousquet E et al. 1998. Efficacy of doxycycline in feed for the control of pneumonia caused by *Pasteurella multocida* and *Mycoplasma hyopneumoniae* in fattening pigs. *Vet Rec* 143:269.
- Bottcher T et al. 2004. Clindamycin is neuroprotective in experimental *Streptococcus pneumoniae* meningitis compared with ceftriaxone. *J Neurochem* 91:1450.
- Brouillette E et al. 2004. Mouse mastitis model of infection for antimicrobial compound efficacy studies against intracellular and extracellular forms of *Staphylococcus aureus*. *Vet Microbiol* 101:253.
- Butts JD. 1994. Intracellular concentrations of antibacterial agents and related clinical implications. *Clin Pharmacokinet* 27:63.
- Carbon C. 1998. Pharmacodynamics of macrolides, azalides and streptogramins: effect on extracellular pathogens. *Clin Infect Dis* 27:28.
- Carcas AJ et al. 1999. Tobramycin penetration into epithelial lining fluid of patients with pneumonia. *Clin Pharmacol Ther* 65:245.
- Cazzola M et al. 2002. Delivering antibacterials to the lungs: considerations for optimizing outcomes. *Am J Respir Med* 116:1261.
- Cerca N et al. 2005. Comparative assessment of antibiotic susceptibility of coagulase-negative staphylococci in biofilms versus planktonic culture as assessed by bacterial enumeration or rapid XTT colorimetry. *J Antimicrob Chemother* 56:331.
- Cogan NG et al. 2005. Modeling physiological resistance in bacterial biofilms. *Bull Math Biol* 67:831.
- Constable PD, Morin DE. 2002. Use of antimicrobial susceptibility testing of bacterial pathogens isolated from the milk of dairy cows with clinical mastitis to predict response to treatment with cephalosporins and oxytetracycline. *J Am Vet Med Assoc* 221:103.
- Costerton JW et al. 1999. Bacterial biofilms: A common cause of persistent infections. *Science* 284:1318.
- Craig WA. 1993. Post-antibiotic effects in experimental infection models: relationship to in vitro phenomena and to treatment of infections in man. *J Antimicrob Agents* 31, Suppl D, 149.
- Craig WA. 2004. U.S. Food and Drug Administration Center for Drug Evaluation and Research. Overview of use of PK-PD in streamlining drug development. http://www.fda.gov/cder/drug/antimicrobial/FDAIDSAISAPresentation/Dr.%20Craig_files/frame.htm.
- Craig WA et al. 2004. The inoculum effect: fact or artifact? *Diagn Microbiol Infect Dis* 2004 50:229.
- Craig, WA, Dalhoff A. 1998. Pharmacodynamics of fluoroquinolones in experimental animals. In: *Handbook of Experimental Pharmacology*, ed. Born GVR et al., 208-232. Berlin: Springer Verlag.
- Cucarella C et al. 2004. Role of biofilm-associated protein bap in the pathogenesis of bovine *Staphylococcus aureus*. *Infect Immun* 72:2177.
- Dalhoff A, Shalif I. 2003. Immunomodulatory effects of quinolones. *Lancet Inf Dis* 3:359.
- Deziel MR et al. 2005. Effective antimicrobial regimens for use in humans for therapy of *Bacillus anthracis* infections and postexposure prophylaxis. *Antimicrob Agents Chemother* 49:5099.
- Drusano GL. 2003. The use of Monte-Carlo simulations in antibacterials: sense and non-sense. 12th ISAP Co-sponsored Symposium, Glasgow, UK. <http://www.isap.org/2003/ISAP-ECCMID-Glasgow/intro.htm>.
- Drusano GL. 2004. Antimicrobial pharmacodynamics: critical interactions of 'bug and drug'. *Nat Rev Microbiol* 4:289.
- Drusano GL et al. 1993. Pharmacodynamics of a fluoroquinolone antimicrobial agent in a neutropenic rat model of *Pseudomonas* sepsis. *Antimicrob Agents Chemother* 37:483.
- Drusano GL et al. 2001. Use of preclinical data for selection of a phase II/III dose for evernimicin and identification of a preclinical MIC breakpoint. *Antimicrob Agents Chemother* 45:13.
- Drusano GL et al. 2004. Relationship between fluoroquinolone area under the curve: minimum inhibitory concentration ratio and the probability of eradication of the infecting pathogen, in patients with nosocomial pneumonia. *J Infect Dis* 189:1590.
- Drusano GL, Preston SL. 2002. Utility of an 800 mg dose of telithromycin for community-acquired pneumonia caused by extracellular pathogens: an assessment by pharmacodynamic modeling and Monte Carlo simulation. Abstract 1364, 12th European Congress of Clinical Microbiology and Infectious Diseases. Milan, Italy.
- Dudley MN, Ambrose PG. 2000. Pharmacodynamics in the study of drug resistance and establishing in vitro susceptibility breakpoints: ready for prime time. *Current Opin Microbiol* 3:515.
- FDA Center for Veterinary Medicine. 2004. Tulathromycin solution for parenteral injection for treatment of bovine and swine respiratory diseases: microbiological effects on bacteria of human health concern. www.fda.gov/cvm/Documents/Tulathromycin.pdf
- Feder HM Jr. 1986. Chloramphenicol: what we have learned in the last decade. *South Med J* 79:1129.
- Finberg RW et al. 2004. The importance of bactericidal drugs: future directions in infectious disease. *Clin Infect Dis* 39:1314.
- Firsov AA et al. 1998a. A new approach to in vitro comparisons of antibiotics in dynamic models: equivalent area

- under the curve /MIC breakpoints and equiefficient doses of trovafloxacin and ciprofloxacin against bacteria of similar susceptibilities. *Antimicrob Agents Chemother* 42:2841.
- Firsov AA et al. 1998b. Inter- and intraquinolone predictors of antimicrobial effect in an in vitro dynamic model: new insight into a widely used concept. *Antimicrob Agents Chemother* 42:659.
- Firsov AA et al. 1999. Prediction of the effects of inoculum size on the antimicrobial action of trovafloxacin and ciprofloxacin against *Staphylococcus aureus* and *Escherichia coli* in an in vitro dynamic model. *Antimicrob Agents Chemother* 43:498.
- Forrest A et al. 1993. Pharmacodynamics of intravenous ciprofloxacin in seriously ill patients. *Antimicrob Agents Chemother* 37:1073.
- Frimodt-Møller N. 2002. Correlation between pharmacokinetic/pharmacodynamic parameters and efficacy for antibiotics in the treatment of urinary tract infection. *J Antimicrob Agents* 19:546.
- Frimodt-Møller N et al. 1981. Effect of urine concentration versus tissue concentration on ampicillin and mecillinam on bacterial adherence in the rat bladder. *Invest Urol* 18:322.
- Gips M, Soback S. 1999. Norfloxacin pharmacokinetics in lactating cows with sub-clinical and clinical mastitis. *J Vet Pharmacol Ther* 22(3):202.
- Goscinski G et al. 2004. Endotoxin neutralization and anti-inflammatory effects of tobramycin and ceftazidime in porcine endotoxin shock. *Crit Care* 8: R35.
- Gunderson BW et al. 2001. What do we really know about antibiotic pharmacodynamics? *Pharmacother* 21(10 pt2):302S.
- Honeybourne D et al. 1994. The levels of clarithromycin and its 14-hydroxy metabolite in the lung. *Eur Respir J* 7:1275.
- Ibrahim KH et al. 2002. Microbiologic effectiveness of time- or concentration-based dosing strategies in *Streptococcus pneumoniae*. *Diagn Microbiol Infect Dis* 44:265.
- Jumbe N et al. 2003. Application of a mathematical model to prevent in vivo amplification of antibiotic-resistant bacterial populations during therapy. *J Clin Invest* 112:275.
- Keren I et al. 2004a. Persister cells and tolerance to antimicrobials. *FEMS Microbiol Lett* 230:13.
- Keren I et al. 2004b. Specialized persister cells and the mechanism of multidrug tolerance in *Escherichia coli*. *J Bacteriol* 186: 8172.
- König C et al. 1998. Bacterial concentrations in pus and infected peritoneal fluid—implications for bactericidal activities of antibiotics. *J Antimicrob Chemother* 42: 227.
- König C et al. 2001. Factors compromising antibiotic activity against biofilms of *Staphylococcus epidermidis*. *Eur J Clin Microbiol Infect Dis* 20:20.
- Kumon H. 2000. Management of biofilm infections in the urinary tract. *World J Surg* 24:1193.
- Labro M-T. 2000. Interference of antibacterial agents with phagocyte functions: immunomodulation or “immunofairy tales”. *Clin Microbiol Rev* 13:615.
- Levison ME. 2004. Pharmacodynamics of antimicrobial drugs. *Infect Dis Clin N Am* 18:451.
- Lister PD, Sanders CC. 2001. Pharmacodynamics of moxifloxacin, levofloxacin and sparfloxacin against *Streptococcus pneumoniae*. *J Antimicrob Chemother* 47:811.
- Lister PD. 2001. Pharmacodynamics of moxifloxacin and levofloxacin against *Staphylococcus aureus* and *Staphylococcus epidermidis* in an in vitro pharmacodynamic model. *Clin Infect Dis* 15:32 Suppl 1:S33.
- Liu P et al. 2005. Pharmacokinetic-pharmacodynamic modelling of antibacterial activity of cefpodoxime and cefixime in in vitro kinetic models. *Int J Antimicrob Agents* 25:120.
- Lode H et al. 1998. Pharmacodynamics of fluoroquinolones. *Clin Infect Dis* 127:33.
- MacGowan AP et al. 2001a. Pharmacodynamics of gemifloxacin against *Streptococcus pneumoniae* in an in vitro pharmacokinetic model of infection. *Antimicrob Agents Chemother* 45:2916.
- MacGowan A, Bowker K. 2002. Developments in PK/PD: optimizing efficacy and prevention of resistance. A critical review of PK/PD in in vitro models. *Int J Antimicrob Agents* 19:291.
- Martinez MN, Amidon GL. 2002. A mechanistic approach to understanding the factors affecting drug absorption: a review of fundamentals. *J Clin Pharmacol* 42:620.
- McDonald PJ et al. 1981. Post-antibiotic leukocyte enhancement: increased susceptibility of bacteria pretreated with antibiotics to activity of leukocytes. *Rev Infect Dis* 3: 38.
- Mizunaga S et al. 2005. Influence of inoculum size of *Staphylococcus aureus* and *Pseudomonas aeruginosa* on in vitro activities and in vivo efficacy of fluoroquinolones and carbapenems. *J Antimicrob Chemother* 56:91.
- Morrissey I, George JT. 1999. The effect of inoculum size on bactericidal activity. *J Antimicrob Chemother* 43:423.
- Morrison DC. 1998. Antibiotic-mediated release of endotoxin and the pathogenesis of gram-negative sepsis. *Prog Clin Biol Res* 397:199.
- Mouton JW et al. 2005. Standardization of pharmacokinetic/pharmacodynamic (PK/PD) terminology for anti-infective drugs: an update. *J Antimicrob Chemother* 55:601.
- Mouton JW, Vinks AA. 2005a. Pharmacokinetic/pharmacodynamic modelling of antibacterials in vitro and in vivo using bacterial growth and kill kinetics: the minimum inhibitory concentration versus stationary concentration. *Clin Pharmacokinet* 44:201.
- Mouton JW, Vinks AA. 2005b. Relationship between minimum inhibitory concentration and stationary concentration revisited: growth rates and minimum bactericidal concentrations. *Clin Pharmacokinet* 44:767.
- Mueller M et al. 2004. Minireview: Issues in pharmacokinetics and pharmacodynamics of anti-infective agents: distribution in tissue. *Antimicrob Agents Chemother* 48:1441.
- Nightingale CH et al. 2000. Pharmacodynamics and pharmacokinetics of levofloxacin. *Chemother* 46 Suppl 1: 6.

- Nolan CM, Ulmer WC Jr. 1980. A study of cephalothin and desacetylcephalothin in cerebrospinal fluid in therapy for experimental pneumococcal meningitis. *J Infect Dis* 141:326.
- Oliver RE et al. 2001. A whole-body physiologically based pharmacokinetic model incorporating dispersion concepts: short and long time characteristics. *J Pharmacokinetics Pharmacodynamics* 28:27.
- Otto M. 2004. Virulence factors of the coagulase-negative staphylococci. *Front Biosci* 9:841.
- Owens WE et al. 1997. Comparison of success of antibiotic therapy during lactation and results of antimicrobial susceptibility tests for bovine mastitis. *J Dairy Sci* 80:313.
- Potera C. 1999. Forging a link between biofilms and disease. *Science* 283:1837.
- Preston SL et al. 1998. Pharmacodynamics of levofloxacin: a new paradigm for early clinical trials. *J Am Med Assoc* 279:125.
- Prins JM et al. 1994. Clinical relevance of antibiotic-induced endotoxin release. *Antimicrob Agents Chemother* 38:1211.
- Reato G et al. 2004. Immunomodulating effect of antimicrobial agents on cytokine production by human polymorphonuclear neutrophils. *Int J Antimicrob Agents* 23:150.
- Ryan DM. 1993. Pharmacokinetics of antibiotics in natural and experimental superficial compartments in animals and humans. *J Antimicrob Chemother* 31 Suppl D:1.
- Sánchez-Navarro A et al. 2001. A retrospective analysis of pharmacokinetic-pharmacodynamic parameters as indicators of the clinical efficacy of ceftizoxime. *Clin Pharmacokinetics* 40:125.
- Sato A et al. 1997. Effect of 6-fluoro-8-methoxy quinolone (AM-1155) against chronic infection with *Pseudomonas aeruginosa* in a rat model. *J Antimicrob Chemother* 44:337.
- Schilling JD, Hultgren SJ. 2002. Recent advances into the pathogenesis of recurrent urinary tract infections: the bladder as a reservoir for uropathogenic *Escherichia coli*. *Int J Antimicrob Agents* 19:451.
- Scorneaux B, Shryock TR. 1999. Intracellular accumulation, subcellular distribution and efflux of tilmicosin in bovine mammary, blood, and lung cells. *J Dairy Sci* 82:1202.
- Shryock TR et al. 1998. The effects of macrolides on the expression of bacterial virulence mechanisms. *J Antimicrob Chemother* 41:505.
- Siebert GA et al. 2004. Ion-trapping, microsomal binding, and unbound drug distribution in the hepatic retention of basic drugs. *J Pharm Exp Therap* 308:228.
- Skálová L et al. 2003. Reduction in flobufen in pig hepatocytes: effect of pig breed (domestic, wild) and castration. *Chirality* 15:213.
- Slocumbe RF et al. 1985. Importance of neutrophils in the pathogenesis of acute pneumonia pasteurellosis in calves. *AM J Vet Res* 46:2253.
- Stevens DL et al. 1995. Antibiotic effects on bacterial viability, toxin production, and host response. *Clin Infect Dis* 20(Suppl 2):S154.
- Thomson Corporation. Thomson Healthcare PDR® Electronic Library. <http://www.thomsonhc.com/pdrel/librarian>.
- Toothaker RD, Welling PG, Craig WA. 1982. An in vitro model for the study of antibacterial dosage regimen design. *J Pharm Sci* 71(8):861.
- Toutain PL et al. 2002. The pharmacokinetic-pharmacodynamic approach to a rational dosage regimen for antibiotics. *Res Vet Sci* 73:105.
- Toutain PL. 2002. Pharmacokinetic/pharmacodynamic integration in drug development and dosage-regimen optimization for veterinary medicine. *AAPS Pharm Sci* 4(4):38.
- Toutain PL. 2005. The role of PK/PD in veterinary drug development. In: *Proceedings, American Academy of Veterinary Pharmacology and Therapeutics* <http://www.avis.org/aavpt>.
- Uemura R et al. 2004. Effect of antimicrobial agents on the production and release of shiga toxin by enterotoxaemic *Escherichia coli* isolates from pigs. *J Vet Med Sci* 66:899.
- US FDA Center for Veterinary Medicine. 2004. Tulathromycin Solution for Parenteral Injection for Treatment of Bovine and Swine Respiratory Diseases. Microbiological Effects on Bacteria of Human Health Concern: a Qualitative Risk Estimation. www.fda.gov/cvm/Documents/Tulathromycin.pdf
- Van den Broek PJ. 1989. Antimicrobial drugs, microorganisms, and phagocytes. *Rev Infect Dis* 11:213.
- Wright DW et al. 2000. Application of fluoroquinolone pharmacodynamics. *J Antimicrob Chemother* 46:669.
- Yata N et al. 1990. Phosphatidylserine as a determinant for the tissue distribution of weakly basic drugs in rats. *Pharm Res* 7:1019.
- Zelenitsky SA et al. 2003. AUC₀₋₁/MIC is a continuous index of fluoroquinolone exposure and predictive antibacterial response for *Streptococcus pneumoniae* in an in vitro infection model. *J Antimicrob Chemother* 51:905.
- Zhanell GG et al. 2001. In vitro pharmacodynamic modelling simulating free serum concentrations of fluoroquinolones against multidrug-resistant *Streptococcus pneumoniae*. *J Antimicrob Chemother* 47:435.
- Zorko M et al. 2005. Combination of antimicrobial and endotoxin-neutralizing activities of novel oleoylamines. *Antimicrob Agents Chemother* 49:2307.

Principles of Antimicrobial Drug Selection and Use

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The aim of antimicrobial therapy is to assist the host's specific and non-specific defense mechanisms in containing and eliminating invading microorganisms. The ability to do this is enhanced when therapeutic drug concentrations are rapidly produced at the site of infection and maintained for a sufficient length of time. In doing so the pathogen's ability to replicate is reduced or eliminated, thus also decreasing the production of toxic substances, both from the host and the pathogen. The overall result is elimination of the infection with a decrease in the disruption of function of adjacent tissues and acceleration of the host's return to health.

Antimicrobial therapy involves a calculated risk that selective toxicity of the drug for the microorganism will succeed before any toxic effect of the drug on the host occurs. A requirement of all drug therapy is also that it be rational. With the increasing choice of a wide array of highly effective antimicrobial drugs, with dosages based on pharmacokinetic analysis of drug disposition in the species of interest, and with selection of the appropriate drug based on clinical microbiological data and pharmacodynamic indices, rational antimicrobial therapy is more applicable today than in the history of antimicrobial therapy.

The considerations affecting the choice of an antimicrobial drug are illustrated in Figure 6.1.

Risks Associated with Antimicrobial Treatment

Antimicrobial agents can have a wide variety of damaging effects, including: (1) direct host toxicity, (2) adverse interactions with other drugs, (3) interference with the protective effect of normal host microflora or disturbance of the metabolic function of microbial

flora in the digestive tract of herbivores, (4) selection or promotion of antimicrobial resistance, (5) tissue necrosis at injection sites, (6) drug residues in animal products that are intended for human consumption, (7) impairment of the host's immune or defense mechanisms, and (8) damage to fetal or neonatal tissues.

Direct Host Toxicity

Direct host toxicity is the most important factor limiting drug dosage. The selective toxicity of antimicrobials is variable. Some agents, such as beta-lactams, are generally considered to be safe, whereas others, such as the aminoglycosides, are potentially toxic. Antimicrobial drugs can damage the function of many organs or tissues, particularly the kidneys (e.g., aminoglycosides, amphotericin B); nervous system (e.g., aminoglycosides, polymyxins); liver (e.g., tetracyclines, chloramphenicol); heart (e.g., aminoglycosides, monensin, and tetracyclines); immune system (e.g., penicillin G); hematopoietic system (e.g., sulfa drugs, chloramphenicol); retina (e.g., fluoroquinolones); and joint cartilage (e.g., fluoroquinolones). The toxicity of antibiotics with a narrow margin of safety can be minimized by using the lowest effective doses and the shortest duration of treatment, by substituting equally effective but less toxic agents, or by using a combination of antimicrobial agents that work synergistically against the pathogen without increased toxicity to the host.

Drug Interactions Involving Antimicrobial Agents

Adverse drug interactions can occur in many ways, both in vitro and in vivo, and should be anticipated. These interactions can affect intestinal absorption, enhance or slow liver metabolism, interfere with kidney excretion, or result in competition for receptors or plasma proteins. Examples are shown in Table 6.1.

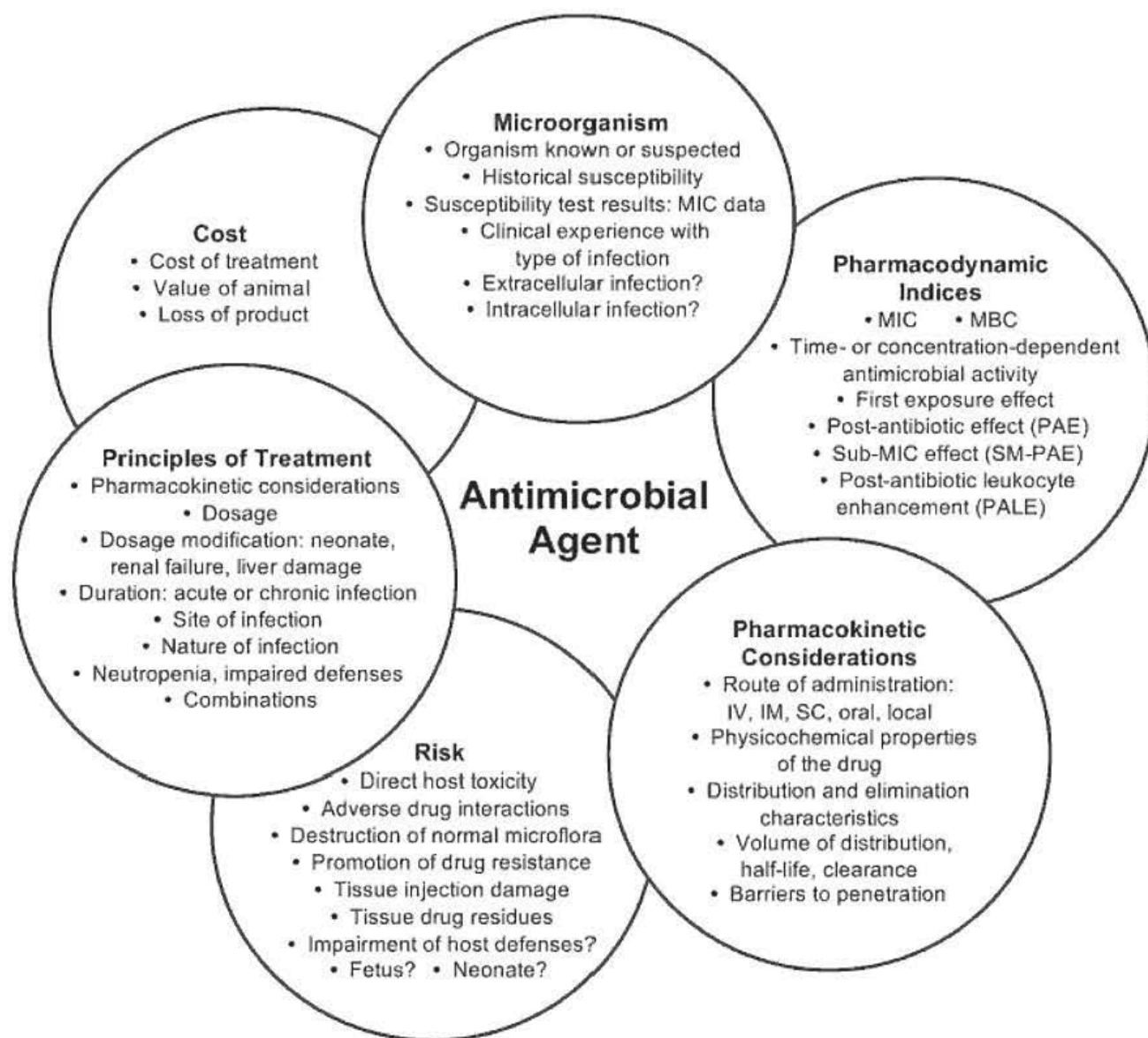


Figure 6.1. Some considerations in selection and use of antimicrobial drugs.

Absorption from the intestine may be affected non-specifically by food, or through pH, fat, or ionic chelating effects (i.e., divalent or trivalent cations). The influence of food on oral absorption of some antibiotics is summarized in Table 6.2. Antibiotics may also affect liver microsomal enzymes. Notable examples are described in sections on individual drugs. In the kidney, the pH of the urine may, depending on the pKa of the drug, affect the excretion and absorption of weak acids and weak bases. Many acidic drugs such as penicillins and sulfonamides are secreted actively in the proximal tubules and may interact with other

drugs which are similarly excreted. For example, probenecid has been used for many years to block the active tubular secretion of ampicillin. When probenecid is administered simultaneously with ampicillin, the serum concentration of ampicillin is doubled (Bryskier, 2005).

Drug Incompatibilities

Antimicrobials may be physicochemically incompatible with other agents *in vitro*. For example, tetracyclines are incompatible with any solution containing calcium or magnesium. Although it is appropriate and

Table 6.1. Examples of adverse in vivo drug interactions between antibiotics and other agents.

Antimicrobial Drug	Interacting Drug	Adverse Effect
Aminoglycoside	Cephaloridine, cephalothin, polymyxins, furosemide	Nephrotoxicity
Amphotericin B	Polymyxins, curare-like drugs, anesthetics	Neuromuscular blockade
Chloramphenicol	Aminoglycosides	Nephrotoxicity
Griseofulvin	Dicoumarol, barbiturates	Prolonged anesthesia, anticoagulation
Lincomycin	Dicoumarol, barbiturates	Reduced anticoagulant effect
Monensin	Kaolin-pectate	Decreased lincomycin absorption
Polymyxins	Tiamulin	Neurotoxicity
Rifampin	Aminoglycosides	Nephrotoxicity, neuromuscular blockade
Sulfonamides	Theophylline	Enhanced theophylline clearance
Tetracyclines	Oral anticoagulants	Prolonged anticoagulant effect
	Barbiturates	Anesthetic potentiation
	Oral iron, calcium, magnesium	Decreased tetracycline absorption

Table 6.2. Suggested oral administration in relation to feeding.

Better When Fasting ^a	Better With Food	Indifferent to Feeding
Azithromycin	Cefadroxil ^b	Cephalexin ^b
Cephadrine	Chloramphenicol palmitate ^d	Chloramphenicol capsules, tablets ^{b,d}
Most erythromycin preparations ^b	Doxycycline ^e	Chloramphenicol palmitate ^b
Fluoroquinolones ^c	Griseofulvin	Clarithromycin ^b
Isoniazid	Itraconazole	Ethambutol
Lincomycin	Ketoconazole	Fluconazole
Most penicillins ^b	Metronidazole ^e	Hetacillin
Rifampin	Nitrofurantoin ^e	Spiramycin ^f
Most sulfonamides		
Most tetracyclines		

Source: Data are from human studies, except as indicated.

^aAbsorption of these drugs may be reduced or delayed in ingesta. Fasting means no food for 1 to 2 hours before and 1 to 2 hours after dosing.

^bCanine data.

^cEnrofloxacin availability is reduced in ingesta in dogs. Effects of ingesta on fluoroquinolones are considered generally mild, but milk and yogurt should be avoided.

^dFeline data.

^eFood may reduce gut irritation without hindering absorption significantly.

^fHuman data. Porcine data indicate better when fasting.

common practice to use a combination of a cephalosporin and an aminoglycoside in vivo, many cephalosporins are not compatible with aminoglycosides in suspension. Thus, it is not a good practice to mix antimicrobial agents in the same vessel. The lack of an obvious interaction, e.g., precipitate, does not mean a chemical inactivation has not occurred.

Antibiotics and the Immune System

Antimicrobial drugs may enhance or suppress host defenses (See Chapter 5 for a more detailed description). These effects may be associated with alterations in cy-

tokine production or the production of other inflammatory intermediates. The complexity of the interactions of antimicrobial drugs with bacteria and phagocytic cells is illustrated in Figure 5.2. It is well known that microorganisms that have been damaged by antimicrobial drugs are more susceptible to killing by phagocytes. The ability of some antibiotics to penetrate and to concentrate within cells, particularly phagocytic cells, while not guaranteeing efficacy, is an important consideration in the treatment of intracellular bacterial infections. For example, phagocyte alteration of pathogen metabolism or structure may render the

pathogen more susceptible to the effect of the antimicrobial agent, and drug concentrations too low to have a cidal effect may render the microbe more susceptible to leukocyte action, an event associated with post-antibiotic leukocyte enhancement (McDonald et al., 1981).

Factors Determining Choice of Antibiotic

Appropriate antimicrobial chemotherapy requires the attending clinician to have a reasonable idea as to the pathogen(s) involved in the infectious disease process and the ability of the chosen antimicrobial agents to reach therapeutic concentrations at the site of infection. While clinical experience may aid the veterinarian in suspecting a given etiological agent, it is optimal to obtain samples for culture and susceptibility testing in order to select the most appropriate drug and dose to use. Samples for bacteriologic culture should be collected from the actual site of infection before administering an antimicrobial drug. A Gram stain of an appropriately collected sample may provide insight as to the etiological agent, but in many cases it is necessary to isolate and identify the pathogen and determine its susceptibility profile.

In certain situations, antibacterial therapy is begun before a specific bacterial pathogen has been identified. The choice of agent is guided by the results of studies identifying the most common pathogens at a given site or in that clinical setting, by pharmacodynamic considerations, and by knowing the resistance profile of the expected pathogen in a particular hospital or geographic area. Situations in which empirical antimicrobial therapy is appropriate include:

- (1) Life-threatening infections: Suspected bacterial infections in an animal with a life-threatening illness should be treated empirically while awaiting culture and susceptibility results. Unless the clinical disease is characteristic for a specific microorganism, it is common practice to initiate therapy with one or more antimicrobial agent(s) to provide broad-spectrum coverage. Therapy is later tailored to address the specific susceptibilities of the pathogen cultured.
- (2) Treatment of mild infections in unhospitalized patients: In many situations it is appropriate to treat individual animals with non-life-threatening

infections without obtaining culture. However, if the infection recurs or fails to respond to initial therapy, efforts should be made to obtain a proper sample for culture to guide re-treatment. When many animals are affected by the same disease, it is preferable to obtain samples for culture and susceptibility from at least a few affected animals.

The selection of an antimicrobial agent depends on: (1) the likely identity of the infecting microorganism(s) at a given site of infection, (2) knowledge of the usual susceptibility profile of the suspected pathogen(s), (3) knowledge of factors that affect drug concentration at the site of infection, (4) knowledge of drug toxicity and factors that enhance it, (5) the cost of treatment, and (6) regulations about drug use including drug withdrawal times where applicable.

Bacterial Susceptibility

Antimicrobial susceptibility of some bacterial pathogens, notably beta-hemolytic streptococci and *Arcanobacterium pyogenes*, is predictable to certain antimicrobial agents, e.g., benzyl penicillins (Chapter 2). This is not the case for most Gram-negative bacteria that readily acquire resistance genes (Chapter 3). Every veterinary practice should have access to a laboratory capable of determining the antimicrobial susceptibility of bacterial pathogens. For properly collected and transported samples, a laboratory should be able to provide information regarding pathogen identification and susceptibility within 48 hours of receipt. As critical as the identification of the pathogen is the assurance that the susceptibility testing has been done appropriately. Improperly conducted susceptibility tests can result in a resistant organism being reported as susceptible and vice versa (Chapter 2).

Laboratory results may be misleading for several reasons, including: (1) failure to isolate the causative agent due to poor sample collection or transport (e.g., anaerobes were involved in the infection but died out due to aerobic transport); (2) misinterpretation of the significance of normal flora for such reasons as inexperience of the laboratory personnel, poor sample collection and transport, or an error in interpreting the laboratory results by the submitting clinician; or (3) inappropriate antimicrobial susceptibility testing. It is not uncommon for a laboratory to overlook the importance of running appropriate quality control or-

ganisms when performing antimicrobial susceptibility tests. Failure to do so could result in misleading or erroneous susceptibility results. Detailed discussion of in vitro antimicrobial susceptibility testing is provided in Chapter 2.

Choice of Antimicrobial Drugs

The ideal drug is one to which the pathogen is susceptible, reaches effective concentration at the site of infection, is nontoxic to the host, requires minimal stress to the animal, and is inexpensive. While benzyl penicillin may fit these criteria when treating a skin infection in a dog caused by *Streptococcus canis*, few infections are this simple. To assist the veterinarian in choosing the appropriate antimicrobial agent, a laboratory should provide as much information as possible. Along these lines, the most basic information the laboratory can provide is qualitative susceptibility (susceptible, intermediate, or resistant, SIR) results. Quantitative (MIC) results may be more useful than the traditional SIR data because MIC data define more precisely the degree of susceptibility of the pathogen. Armed with this information, the clinician can more precisely define the dosing regimen that fits the criteria listed above. In making the decision as to which drug to use, the clinician should also keep in mind that bactericidal drugs are required: (1) for serious life-threatening infections; (2) when host defenses are seriously impaired; (3) for infections of vital tissues such as the central nervous, cardiovascular, and skeletal systems, where host defenses may not be fully functional; and (4) in immunodeficient or immunosuppressed animals. For infection of a less severe nature, bacteriostatic agents may be as or more useful than bactericidal drugs.

Where appropriate, a narrow-spectrum drug may be more appropriate than a broad-spectrum antibacterial because the narrow spectrum is less likely to target normal microbial flora. In this regard, pharmacokinetic considerations are also relevant. For example, drugs excreted via the bile may disturb the intestinal flora more than those excreted via the kidneys. Drug combinations should be considered in seriously ill patients with severe infections when results of bacteriologic tests are not available. The availability of a dosage form of the antimicrobial drug of choice that is suitable for administration to the particular species of animal is another factor influencing the final choice of antimicrobial drug.

Principles of Antimicrobial Treatment

To some extent, drug dosage can be tailored to the susceptibility of the organism, the site of infection, and the pharmacokinetic and pharmacodynamic properties of the selected antimicrobial agent. However, it should be recognized that in vitro susceptibility data are laboratory-derived, and the standardized conditions under which the susceptibility data was generated do not exist at the site of infection. It is also important to recognize that pharmacokinetic data represent mean values obtained from different animals and that the immune status of the host, as well as its physiological and psychological status, can influence the therapeutic outcome.

Factors involved in tailoring a dosing regimen include, among other things, the susceptibility of the pathogen in terms of MICs, the concentration of the antimicrobial agent at the site of infection in active form (pharmacokinetic properties of the drug), and the pharmacodynamic properties of the antimicrobial agent. These principles are discussed in detail in Chapter 5. Briefly, antimicrobial agents may be categorized as those that exhibit concentration-dependent killing, time-dependent killing, a combination of time-dependent and concentration-dependent killing, and those that are primarily bacteriostatic. Examples of concentrations and time-dependent killing are illustrated in Figure 6.2. For an aminoglycoside such as tobramycin, as the concentration of the antimicrobial agent increases above the MIC of the pathogen (in this case, *Pseudomonas aeruginosa*), the number of viable organisms decreases dramatically. Thus, optimal dosing of concentration-dependent antimicrobial agents involves administration of high doses with long dosing intervals. On the other hand, for a beta-lactam drug such as ticarcillin, the number of viable organisms decreases as concentration of ticarcillin increases from 0.25 of the MIC to one times the MIC to four times the MIC. However, there is very little decrease in viable organisms as the concentration of ticarcillin continues to increase to 16 and 64 times the MIC. Optimal dosing of such antimicrobial agents involves frequent administration. Bacteriostatic agents typically exert time-dependent activity. The relationship between drug concentration and antimicrobial activity is presented in Table 5.5.

Although the factors listed above can contribute to determining the optimal dosage, the factor that most

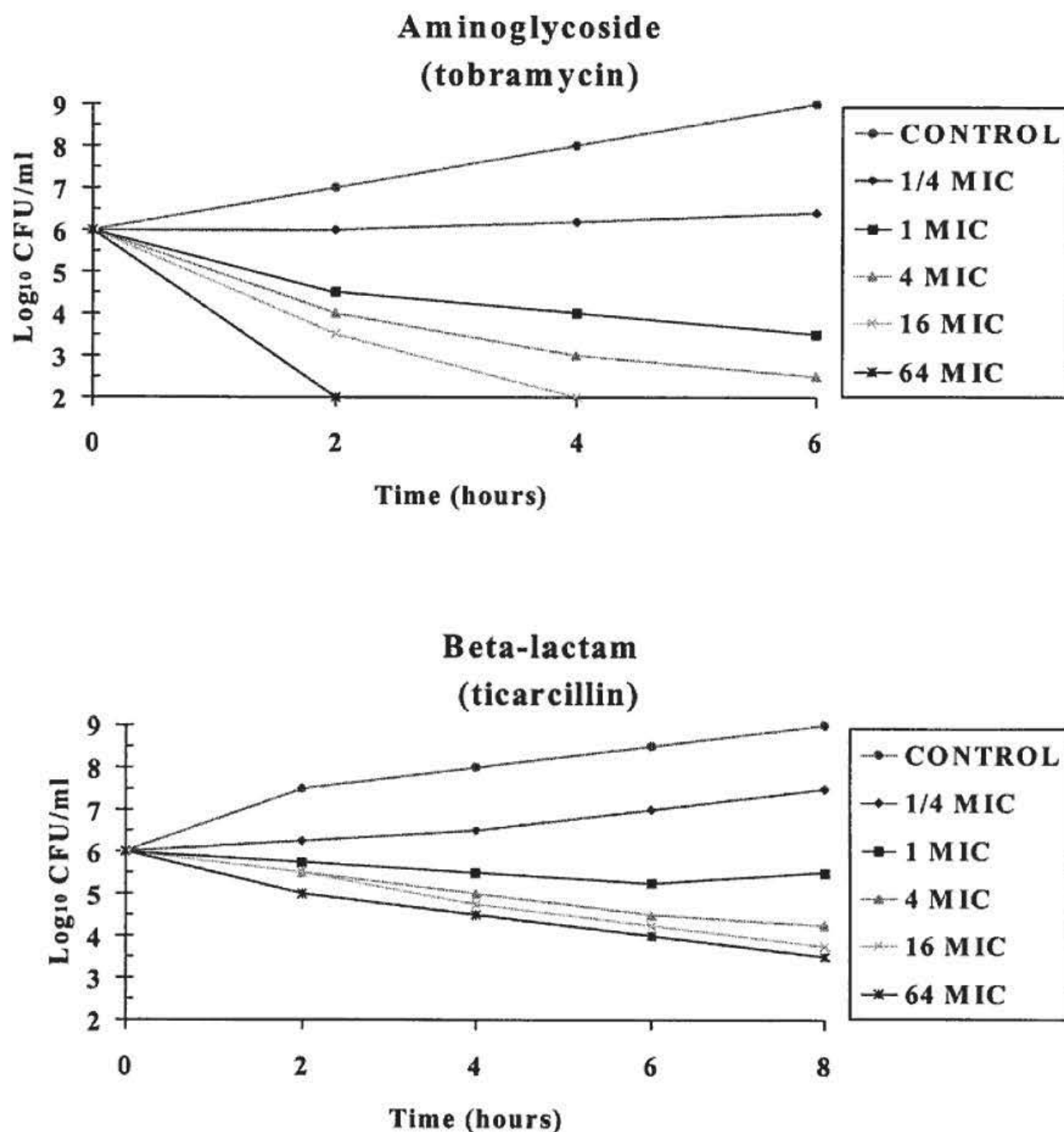


Figure 6.2. Example of concentration-dependent killing by an aminoglycoside (tobramycin). This effect contrasts to the killing by beta-lactams, which depends on the presence of drug concentration above the MIC (time-dependent killing) but is otherwise independent of drug concentration. Reproduced with permission from Craig and Ebert (1991).

frequently limits dosage is toxicity to the host. In most instances the upper level of the recommended dosage should not be exceeded, because this is often determined by toxicity. Sometimes, however, a drug's antibacterial effects may be limiting and may determine

the upper level of dosage. For example, as discussed above, the killing rate of penicillin G (and other beta-lactam drugs) has an optimal concentration, whereas that of the aminoglycosides or fluoroquinolones is proportional to drug concentration. Penicillin G is vir-

tually nontoxic in non-allergic patients, but its dosage is limited by its antibacterial action, whereas the dosage of an aminoglycoside is limited not by antibacterial effects but rather by its toxicity to the host.

Recommended dosing intervals should be followed. With the exception of the penicillins, fluoroquinolones, and aminoglycosides, the interval for IV-administered drugs required to maintain therapeutic plasma concentrations should not usually exceed twice their elimination half-life. Because elimination half-life is based on IV dosing, however, administering appropriate formulations by other routes can be an effective way to lengthen the interval between doses, since absorption may be delayed. For example, a single dose of procaine penicillin G administered IM can maintain effective drug levels for 12 to 24 hours because of slow absorption from the site of administration, even though in all species the elimination half-life of penicillin G is less than one hour. Not following appropriate dosing recommendations is detrimental because the concentration of active drug at the site of infection may not be sufficient to inhibit the pathogen. An example of not following the recommended dosing interval was observed by Waltner-Toews et al. (1986), who reported that calves treated with chloramphenicol once daily were four times more likely to die of pneumonia than calves treated twice daily. Dosages may need to be modified in neonates and in animals with impaired liver or kidney function (Chapter 4).

Duration of Treatment

Although it is universally recognized that a drug must be present at a sufficient concentration for an adequate length of time at the site of infection, the variables affecting length of treatment have not been fully defined. Responses of different types of infections to antimicrobial drugs vary, and clinical experience with many infections is important in assessing response to treatment. For acute infections, it will be clear within two to three days whether or not therapy is clinically effective. If no response is seen by that time, both the diagnosis and treatment should be reconsidered. Treatment of acute infections should be continued for at least two days after clinical and microbiologic resolution of infection. For serious acute infections, treatment should last at least 7 to 10 days. For chronic infections, particularly intracellular infections, treatment will be considerably longer and may, in fact, take months. Some uncomplicated infections, such as cys-

titis in human females, have been treated successfully with single doses of antibiotics, and some antimicrobial agents are now being marketed for single administration in cattle for the treatment of acute respiratory disease. However, in animals, the efficacy of such treatment must be well established before this approach is recommended. Such approaches may, inappropriately, be driven by market competition and cost-efficacy considerations rather than by optimum therapeutic benefit.

Adjunctive Treatment

Adjunctive treatments to antimicrobial therapy are essential in promoting healing. They include debriding necrotic tissues, removing purulent exudate, removing foreign bodies, correcting acid/base and fluid balance, identifying and removing predisposing causes, and providing rest and nursing, when appropriate. It is virtually impossible to treat infection associated with a foreign body without removing it.

Other Considerations

Other considerations in antimicrobial therapy include the cost of the drug and convenience of administration. In food-producing animals, one must know the likelihood of drug residues remaining in tissues or in milk, and the resultant required withdrawal times of agents. For example, aminoglycoside residues can be present in the kidney and liver of cattle for months after administration. Label directions for drug products for food-animal use must be followed or intelligently interpreted. The danger of selecting resistant bacteria by antibiotic use is another important consideration.

Extra-Label Drug Use

Antimicrobial drugs are licensed in many countries only for use for particular purposes at specific dosages, as shown on the manufacturer's product label. Because of the high costs of obtaining approval, many drugs are not approved or may only be approved for narrowly specified purposes at dosages that, in food animals at least, may be more concerned with the potential for drug residues or economics of treatment than with optimal clinical efficacy. In the United States, the Food and Drug Administration's Center for Veterinary Medicine has (with notable exceptions, Chapter 26) a discretionary "extralabel" policy. This means that veterinarians who use drugs in ways not in accord with

label directions, with certain other specifications, will not be prosecuted so long as no illegal tissue residues occur in edible products. The specifications include the need for a careful diagnosis within the context of a valid veterinarian-client-patient relationship and a determination that there are no alternative drugs, that dosage is appropriate, that treated animals are identified, and that extended drug withdrawal ensures no tissue residues. The increasing availability of simple in-house tests for drug residues (Chapter 25) has aided extra-label drug use. For non-food-producing animals, the position is generally that veterinarians may use any legally obtainable antimicrobial drug to treat disease, subject only to subsequent scientific justification before the courts or veterinary licensing body, should this use need to be defended.

Corticosteroid Use

The benefits of using corticosteroids with antimicrobial drugs in the treatment of acute bacterial infections are both controversial and poorly investigated. Clear guidelines are not available. Corticosteroids have many effects on nonspecific and specific host defenses, e.g., suppressing inflammation, impairing phagocytosis, delaying healing, reducing fever, and impairing the immune response. Use of corticosteroids in the treatment of infections would therefore generally be expected to have deleterious effects and should be avoided. However, in the virtual absence of experimental or clinical data supporting their concurrent use, certain circumstances may justify their short-term use: (1) in infections with concurrent life-threatening autoimmune or immune-mediated disorders; (2) in selected extensive, acute local infections to prevent lysosomal enzyme release from neutrophils and resulting tissue destruction; and (3) in the early treatment of meningitis to control inflammation caused by beta-lactam antibiotic induced release of inflammatory mediators and to control cerebral edema (Chapter 22).

Corticosteroids have been used for decades for the treatment of severe sepsis and septic shock, based on their pivotal role in the stress response and their hemodynamic and anti-inflammatory effects. Whereas short-term therapy with high doses of corticosteroids has been ineffective or even harmful in humans with septic shock, prolonged therapy with low doses of hydrocortisone (200-300 mg for 5-7 days or longer) has been shown to have beneficial effects in recently conducted randomized, controlled trials (Chadda et al.,

2004). The role of this modality in the treatment of bacterial sepsis in domestic animals is unknown at this point.

Rapid Attainment of High Tissue Concentrations of Drugs

In acute bacterial infections, especially when using bacteriostatic drugs, it may be useful to administer a priming (loading) dose, usually by giving a high dose by IV injection, to rapidly establish therapeutic drug levels.

Local Administration of Antimicrobial Drugs

Antimicrobial drugs are administered locally in the treatment of a wide variety of infections, including endometritis; skin, outer ear, and wound infections; corneal infections; mastitis; osteomyelitis, septic arthritis and tenosynovitis; and occasionally in bronchopneumonia (by endotracheal or aerosol administration). Local administration has the potential to achieve higher and more persistent drug concentrations than systemic administration. Because of this, local treatments may be administered less frequently than systemic treatments, but this is very site- and drug-dependent. The principles of drug selection and use are those described for systemic antimicrobial drugs, with the caution that the drug vehicle and the drug must not provoke tissue inflammation. For endometritis, local treatment may not penetrate important sites, such as the oviducts or cervix, in comparison to systemic treatment. In the cow and the mare, intrauterine treatment of the involuted uterus often consists of one gram of antibiotic dissolved in 100-250 ml sterile saline administered daily for three to five days, depending on the severity and chronicity of infection. Acute, severe metritis requires systemic antibiotics, which may be supplemented by local treatments.

Endotracheal administration of antibiotics, particularly aminoglycosides, results in high, persistent drug concentrations in the tracheobronchial tree but may be limited in distribution. Because of this, endotracheal administration of antimicrobial agents is generally not recommended except for those tracheobronchial infections that have responded poorly to systemic treatment. Aerosol administration of antimicrobial drugs results in better diffusion throughout the bronchial tree and may have a place in severe infections of the bronchial tract that are unresponsive to other treatments. In some experimental models of pneumonia in mechanically ventilated animals, even

poorly ventilated and consolidated areas of the lungs contained higher antimicrobial drug concentrations after aerosol administration than after IV administration (Goldstein et al., 2002). Nevertheless, the administration of antimicrobial agents by inhalation alone may not be sufficient in patients with severe parenchymal involvement or substantial consolidation. In these cases, aerosol therapy may be more appropriate as an adjunct to systemic administration.

Local delivery of antimicrobial agents is an important adjunct to joint lavage, systemic antimicrobial therapy and, when necessary, surgery in animals with severe infections of the musculoskeletal system (Chapter 22). Intra-articular administration of antimicrobial agents is a common local delivery method in cases of septic arthritis. Regional intravenous or intraosseous infusions are useful alternatives in animals with osteomyelitis of the distal limb, when multiple joints are involved, or when the drug of choice is too irritating for intra-articular use. Antimicrobial-impregnated polymethyl methacrylate for the treatment of osteomyelitis may maintain effective local concentrations of drug for several weeks. Gentamicin-impregnated collagen sponges have also been used successfully in the local treatment of septic arthritis in animals (Chapter 22).

Antimicrobial Drug Combinations

From the earliest days of antibiotic use it was known that combinations of drugs sometimes had synergistic effects where individual agents had failed (Pillai et al., 2005). On the other hand, early studies of the use of a combination of penicillin and chlortetracycline to treat certain types of bacterial meningitis showed that antagonism between drugs might have fatal results. The importance of antagonism is greatest in patients with suppressed immune defenses or severe infections, such as in meningitis, endocarditis, or chronic osteomyelitis. Mechanisms of synergism and antagonism were discussed in Chapter 1. There are four indications for the use of antimicrobial combinations:

(1) **Antimicrobial synergism:** There is a considerable body of literature investigating the role of antimicrobial synergism in the treatment of various bacterial infections in humans and laboratory animals. However, there are surprisingly few examples where in vitro documentation of antimicro-

bial synergism has been predictive of superior clinical activity. In addition to the well documented merit of fixed combinations such as trimethoprim/sulfonamide, combinations of bactericidal agents such as penicillin (or ampicillin or vancomycin) with an aminoglycoside (streptomycin or gentamicin) have proven superior to monotherapy for the treatment of enterococcal endocarditis in humans. Potential advantages of synergistic bactericidal combinations are observed primarily in patients with impaired host defenses.

- (2) **Polymicrobial infections:** Two or more agents may be administered to treat documented or suspected polymicrobial infections (e.g., peritonitis, aspiration pneumonia, female genital tract infections). A classic example is the rat peritonitis model of intestinal perforation in which treatment against both Enterobacteriaceae (e.g., aminoglycoside) and anaerobes (e.g., clindamycin) is necessary to clear infection. The increasing availability of highly active, broad-spectrum, bactericidal drugs has reduced the need for combination therapy in humans. However, in veterinary practice the high cost of many of these newer broad-spectrum drugs is prohibitive, especially in large animal species. As a result, drug combinations are commonly used in veterinary medicine for the treatment of polymicrobial infections.
- (3) **To decrease the emergence of resistant isolates:** The simultaneous use of two or more agents to treat infections caused by bacteria that may develop resistance reduces this likelihood. This is best illustrated in the treatment of tuberculosis in humans, where concurrent therapy with multiple drugs clearly decreases the risk of resistance. This rationale is often discussed for other combinations but it is particularly relevant for rifampin, an agent to which many bacteria develop resistance when used alone.
- (4) **To decrease dose-related toxicity:** Several antimicrobials have dose-related toxicity that may limit their use. Combined therapy may allow dosage reduction of a toxic drug, while ensuring successful therapy. A clinically relevant example is the combination of flucytosine and amphotericin B in the treatment of cryptococcal meningitis, which allows a reduction in the dose of amphotericin B, thereby limiting its toxicity.

Table 6.3. Examples of antimicrobial drug combinations clinically useful in veterinary medicine.

Indication	Drug Combination	Comment
Bovine <i>Staphylococcus aureus</i> mastitis	Penicillin-streptomycin; Ampicillin-clavulanic acid	Synergistic combination
<i>Rhodococcus equi</i> pneumonia of foals	Penicillin-novobiocin	Also approved for streptococcal bovine mastitis
<i>Brucella canis</i> in dogs	Macrolide*-rifampin	Synergistic; prevent emergence of resistance
Peritonitis after intestinal spillage	Minocycline-streptomycin	Synergistic combination
	Gentamicin-clindamycin;	Broad-spectrum antibacterial activity
	Cefuroxime-metronidazole	
Coliform meningitis	Trimethoprim-sulfamethoxazole	Synergistic, good CSF penetration
Cryptococcal meningitis	Amphotericin-flucytosine	Synergistic decreased toxicity
Severe undiagnosed infection	Beta-lactam-gentamicin;	Broad-spectrum, often synergistic combination
	Cefoxitin-clindamycin	

*Azithromycin, clarithromycin, or erythromycin

A few examples of clinically effective combinations used in veterinary medicine are shown in Table 6.3. Combinations should only be used where their efficacy is established.

Combining antimicrobial agents may also have disadvantages. For example, a bacteriostatic drug may neutralize bactericidal effects where these effects are required. Combinations may have additive or synergistic toxicity. They may produce super-infection after destroying normal microbial flora and may have adverse pharmacokinetic interactions. When combination therapy is used, it should be done in such a way as to maximize the synergistic effect. For example, in using an aminoglycoside/beta-lactam combination, the aminoglycoside should be administered once a day for its concentration-dependent killing effect, whereas the beta-lactam should be administered so as to maintain continuous serum concentrations above the MIC of the organism for the majority of the dosing interval.

Failure of Antimicrobial Therapy

Treatment failure has many causes. The antibiotic selected may be inappropriate because of misdiagnosis, poor drug diffusion at the site of infection, inactivity of a given drug at the site of infection (e.g., aminoglycosides in purulent material), failure to identify the etiological agent including inaccurate results of laboratory tests (Chapter 2), resistance of pathogens, intracellular location of bacteria, metabolic state of the pathogen, or errors in sampling. Other factors that may contribute are inadequate dosage or the use of drugs with low bioavailability.

When failure occurs, diagnosis must be reassessed and proper samples collected for laboratory analysis. Patient factors such as the persistence of foreign bodies, neoplasia, and impairment of host defenses are important to consider. It is important also to ensure that persons medicating their own animals comply with dosing instructions.

Drug Withdrawal

Most countries require that antimicrobial drugs not be present in foods for human consumption and specify the time after antibiotic treatment during which animals cannot be slaughtered and milk cannot be sold. These withdrawal periods are specified for different agents (Chapter 25) and extra-label drug use. FARAD (the Food Animal Drug Avoidance Databank) assists veterinarians in estimating residue depletion times for antimicrobial agents that are administered at doses in excess of label recommendations. More information on FARAD may be found in Chapter 25.

Targeted Drug Delivery

Therapeutic efficacy of antimicrobial drugs in vivo may be reduced by their inability to reach the site of infection in adequate amounts. Considerable effort has been devoted to finding ways to target drugs to the appropriate site. One approach has been to encapsulate drugs in liposomes—microscopic, closed lipid vesicles. After IV injection, liposomes are taken up by macrophages in the liver and spleen. Experimentally, liposome-entrapped antimicrobial drugs are more active than conventionally delivered drugs against facultative intracellular pathogens, with the added advan-

tage (for example with amphotericin B) of reduced toxicity. Liposomally entrapped drugs have been used for many years in human medicine, but their use in veterinary medicine is still under investigation.

Bibliography

- Bryskier A. 2005. Penicillins. In: Bryskier A. (ed) *Antimicrobial Agents: Antibacterials and Antifungals*, Washington: ASM Press pp 113-162.
- Craig WA, Ebert SC. 1990. Killing and regrowth of bacteria in vitro: a review. *Scand J Infect Dis Suppl* 74:63.
- Chadda K, Annane D. 2004. The use of corticosteroids in severe sepsis and acute respiratory distress syndrome. *Ann Med*. 34:582.
- Goldstein I, et al. 2002. Lung deposition and efficiency of nebulized amikacin during *Escherichia coli* pneumonia in ventilated piglets. *Am J Respir Crit Care Med* 166:375.
- McDonald PJ, et al. 1981. Post-antibiotic leukocyte enhancement: increased susceptibility of bacteria pretreated with antibiotics to activity of leukocytes. *Rev Infect Dis* 3: 38-44.
- Pillai SK, et al. 2005. Antimicrobial combinations. In: Lorian, V (ed). *Antibiotics in Laboratory Medicine*, 5th Edition. Philadelphia: Lippincott Williams and Wilkins. pp 365-440.
- Waltner-Toews D, et al. 1986. Calf-related drug use on Holstein dairy farms in southwestern Ontario. *Can Vet J*. 27:17.

Section II

Classes of Antimicrobial Agents

Beta-lactam Antibiotics: Penam Penicillins

John F. Prescott

Alexander Fleming's observation in 1929 that colonies of staphylococci were lysed on a plate contaminated with a *Penicillium* mold was the discovery that led to the development of antibiotics. In 1940, Chain and Florey and their associates were the first to produce sufficient quantities of penicillin from cultures of *Penicillium notatum*. Almost a decade later, penicillin G became widely available for clinical use. In clinical application, this antibiotic was found to have limitations which included: relative instability in gastric acid, susceptibility to inactivation by beta-lactamase (penicillinases), and relative inactivity against clinically important Gram-negative bacteria. This inactivity against Gram-negative rods resulted from (1) inability to penetrate the Gram-negative cell wall, (2) lack of available binding sites (penicillin binding proteins), or (3) enzymatic inactivation. Intensive research led to the isolation of the active moiety, 6-aminopenicillanic acid, in the penicillin molecule. This moiety, which consists of a thiazolidine ring (A) attached to a beta-lactam ring (B) that carries a secondary amino group (R-NH-), is essential for antibacterial activity (Figure 7.1). Isolation of the active moiety resulted in the development of semisynthetic penicillins that overcome some but not all of the limitations associated with penicillin G.

The development of the cephalosporin family, which shares the beta-lactam ring with penicillins (Figure 7.2), led to a remarkable array of drugs with varying ability to penetrate different Gram-negative bacterial species and to resist several beta-lactamase enzymes (Chapter 8). Other naturally occurring beta-lactam antibiotics have subsequently been described that lack the bicyclic ring of the classic beta-lactam penicillins and cephalosporins. Many have potent antibacterial activity and are highly inhibitory to beta-

lactamase enzymes. Some, such as the carbapenems, oxacephems, penams, and monobactams, have potent antibacterial activity whereas others, such as the oxapenem clavulanic acid, have no antibacterial activity of their own but possess potent beta-lactamase inhibitory activity (Chapter 9). These latter drugs are combined with older beta-lactams to increase their range of antibacterial activity. Beta-lactam antibiotics are in widespread use because of their selectivity, versatility, and low toxicity.

Chemistry

The penicillins, cephalosporins, carbapenems, monobactams and penams are referred to as beta-lactam antibiotics. Rupture of the beta-lactam ring, which is brought about enzymatically by bacterial beta-lactamases, results in loss of antibacterial activity. Hypersensitivity reactions are associated with the active moieties of the beta-lactam drugs, and because these drugs are of similar structure, caution should be exercised when administering cephalosporins to penicillin-sensitive animals. Substitutions can be made on the beta-lactam ring for specific purposes, such as: (1) increasing resistance to beta-lactamases of clinically important families or species of bacteria; (2) enhancing activity against selected pathogens; or (3) ensuring favorable pharmacokinetic properties. Semisynthetic beta-lactam drugs have been designed for specific purposes.

Mechanism of Action

Beta-lactam antibiotics prevent the bacterial cell wall from forming by interfering with the final stage of peptidoglycan synthesis. They inhibit the activity of the transpeptidase and other peptidoglycan-active enzymes that are called penicillin-binding proteins

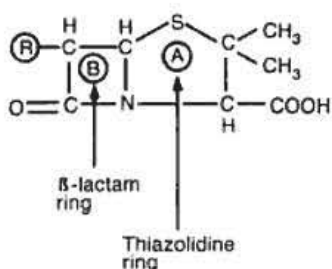


Figure 7.1. Structural formula of penicillin.

(PBPs) (transpeptidases, carboxypeptidases). The PBPs catalyze cross-linkage of the glycopeptide polymer units that form the cell wall. The drugs exert a bactericidal action but cause lysis only of cells which are undergoing active cell wall synthesis. The exact mechanism of lysis is unknown.

Variation in the activity of different beta-lactams re-

sults, in part, from differences in affinity of the PBPs for the drugs. The difference in susceptibility between Gram-positive and Gram-negative bacteria depends on differences in receptor sites (PBPs), the relative amount of peptidoglycan present (Gram-positive bacteria possess far more), the ability of the drugs to penetrate the outer cell membrane of Gram-negative bacteria, and resistance to the different types of beta-lactamase enzymes produced by the bacteria. These differences are summarized in Figures 7.3 and 7.4.

Beta-lactam antibiotics are bactericidal drugs with slower kill rates than aminoglycosides or fluoroquinolones. Killing activity starts after a lag period. Against Gram-positive bacteria, all beta-lactams exhibit an in vitro post-antibiotic effect. This does not carry over for the streptococci in vivo. The beta-lactams do not exhibit a post-antibiotic effect against Gram-negative bacteria, with the possible exception of carbapenems against *Pseudomonas*. Optimal anti-

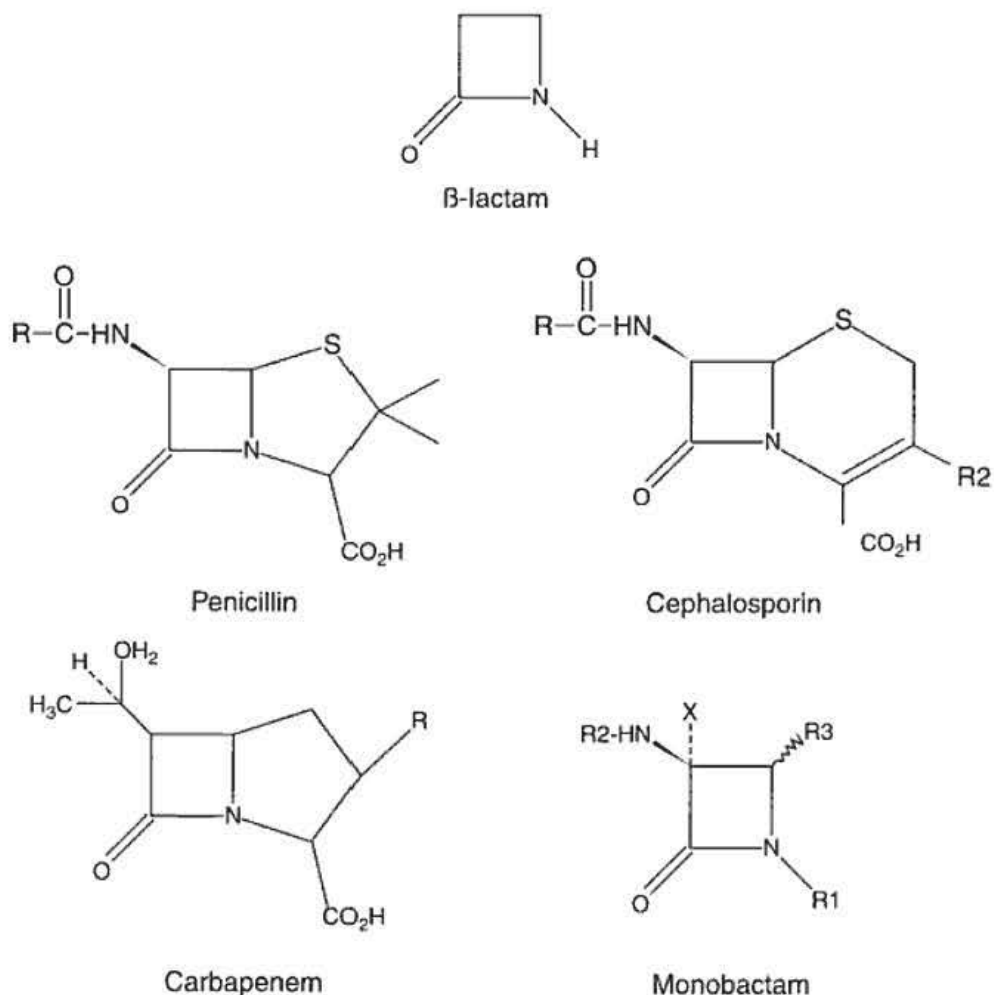


Figure 7.2. Core structures of naturally occurring beta-lactams.

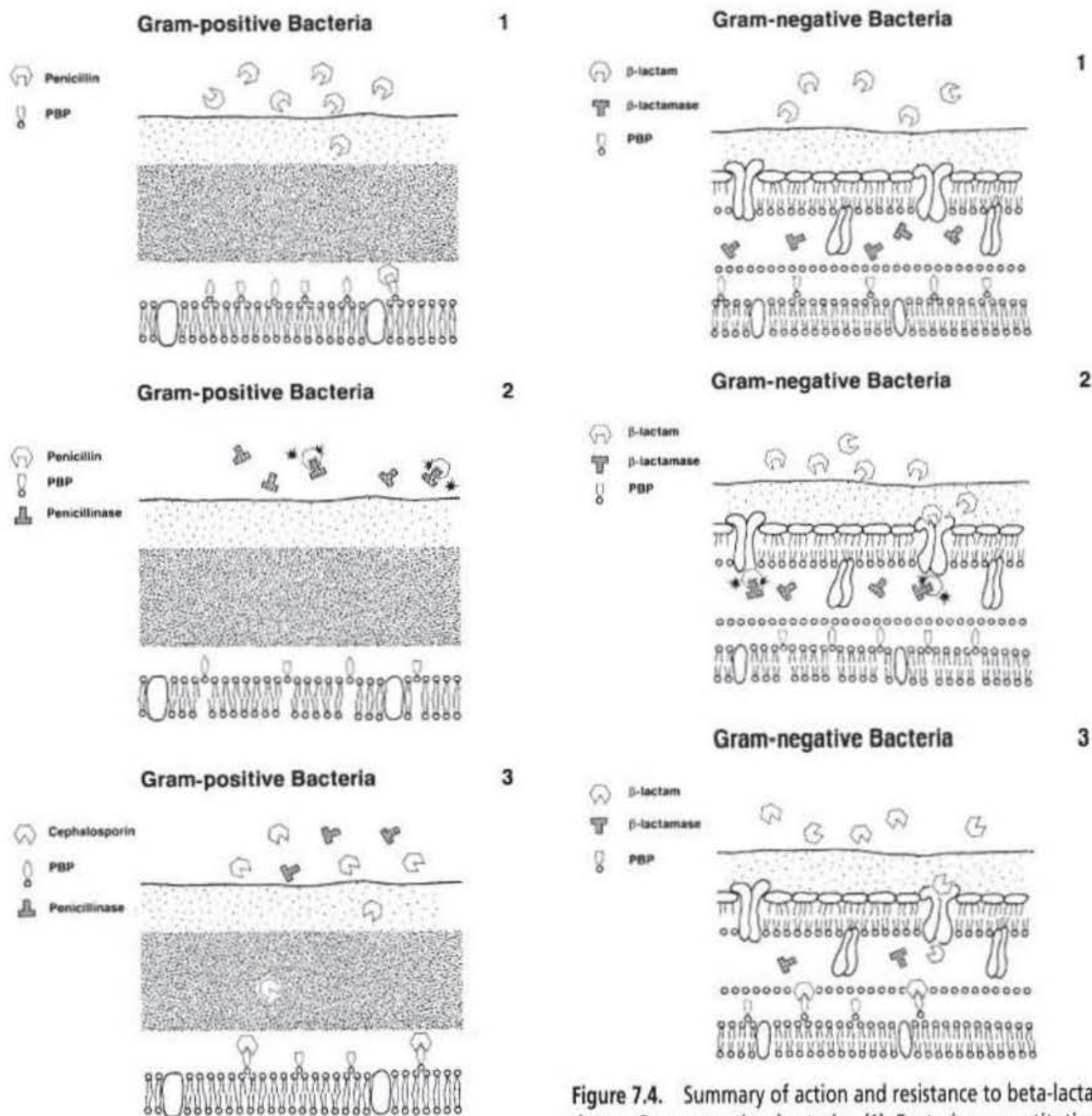


Figure 7.3. Summary of action and resistance to beta-lactam drugs: Gram-positive bacteria. (1) Susceptible bacterium; (2) exogenous beta-lactamase-producing bacterium, e.g., *Staphylococcus aureus*; (3) penicillinase-producing bacterium susceptible to cephalosporin. After Walker, unpublished, with permission.

Figure 7.4. Summary of action and resistance to beta-lactam drugs: Gram-negative bacteria. (1) Bacterium constitutively resistant to penetration by beta-lactam; (2) penetration by beta-lactam but destruction by periplasmic beta-lactamase; (3) susceptible Gram-negative bacterium. After Walker, unpublished, with permission.

bacterial efficacy is time- and not concentration-dependent (Chapters 5 and 6) and requires that serum concentrations exceed the MIC of the pathogen for essentially the entire dosing interval, so that these drugs are best administered frequently or by continuous infusion.

Resistance to Beta-lactam Antibiotics

In Gram-positive bacteria, especially *Staphylococcus aureus*, resistance to penicillin G is mainly through the production of beta-lactamase enzymes that break the beta-lactam ring. *Staphylococcus aureus* secretes beta-lactamase enzymes extracellularly as inducible exoenzymes that are plasmid-mediated (Figure 7.3). The inherent resistance to penicillin G of many Gram-negative bacteria results from low permeability of the Gram-negative cell wall, lack of PBPs, and a wide variety of beta-lactamase enzymes (Figure 7.4). Most Gram-negative bacteria inherently express low levels of species-specific, chromosomally mediated beta-lactamase enzymes within the periplasmic space, which sometimes contribute to resistance. These enzymes hydrolyze susceptible cephalosporins more rapidly than penicillin G, but they hydrolyze ampicillin, carbenicillin, and beta-lactamase-resistant penicillins poorly.

Production of plasmid-mediated beta-lactamases is widespread among common Gram-negative primary and opportunist bacterial pathogens. The enzymes are constitutively expressed, present in the periplasmic space, and cause high-level resistance. The majority are penicillinases rather than cephalosporinases (Figure 7.4). The most widespread are those classified on the basis of their hydrolytic activity as TEM-type beta-lactamases, which readily hydrolyze penicillin G and ampicillin rather than methicillin, cloxacillin, or carbenicillin. The less widespread OXA-type beta-lactamases hydrolyze penicillinase-stable penicillins (oxacillin, cloxacillin, and related drugs). More details on beta-lactamases are given in Chapter 8. Beta-lactamases probably evolved from PBPs as a protective mechanism for soil organisms exposed to beta-lactams in nature, in which they are thought to be widespread through their production by molds. Because of transferable resistance, beta-lactamase production by pathogens is now widespread.

A major advance has been the discovery of broad-spectrum beta-lactamase-inhibitory drugs (e.g., clavulanic acid, sulbactam, tazobactam). These drugs have

weak antibacterial activity but show extraordinary synergism when administered with penicillin G, ampicillin, or amoxicillin because of irreversible binding to the beta-lactamase enzymes of resistant bacteria. Other beta-lactamase inhibitors, such as cefotaxime and carbapenems, have potent antibacterial activity in their own right (Chapter 9).

Bibliography

- Bush K. 2001. New β -lactamases in gram-negative bacteria: diversity and impact on the selection of antimicrobial therapy. *Clin Infect Dis* 32:1085. 1988.
- Juteau J-M, et al. 1991. Molecular distribution of ROB-1 beta-lactamase in *Actinobacillus pleuropneumoniae*. *Antimicrob Agents Chemother* 35:1397

Penam Penicillins

General Considerations

The acidic radical (R), attached to the amino group of 6-aminopenicillanic acid (Figure 7.1), determines the susceptibility of the resulting penicillin to hydrolytic degradation or enzymatic inactivation by bacterial beta-lactamase and the antibacterial activity of the molecule. Both of these factors influence the clinical effectiveness of penicillins, along with the drug concentration attained at the site of infection. The nature of the acidic radical has little influence on the rate of elimination of penicillins, but determines the extent of plasma albumin binding and, to a lesser degree, membrane-penetrating ability. The 6-aminopenicillanic acid moiety and structure of the acid radicals of some penicillins are shown in Figure 7.5.

Penam penicillins are readily distinguished on the basis of antimicrobial activity into six groups ("generations", which largely correspond to their time of introduction into clinical use) (Table 7.1): (1) Benzyl penicillin and its long-acting parenteral forms, (2) Orally absorbed penicillins similar to benzyl penicillin, (3) Staphylococcal penicillinase-resistant isoxazolyl penicillins, (4) Extended or broad-spectrum penicillins, (5) Antipseudomonal penicillins, and (6) Beta-lactamase resistant penicillins.

Since the 1940s, the progressive development of penicillins for clinical use resulted in derivatives with similar activity to benzyl penicillin but which could be administered orally and/or were resistant to *S. au-*

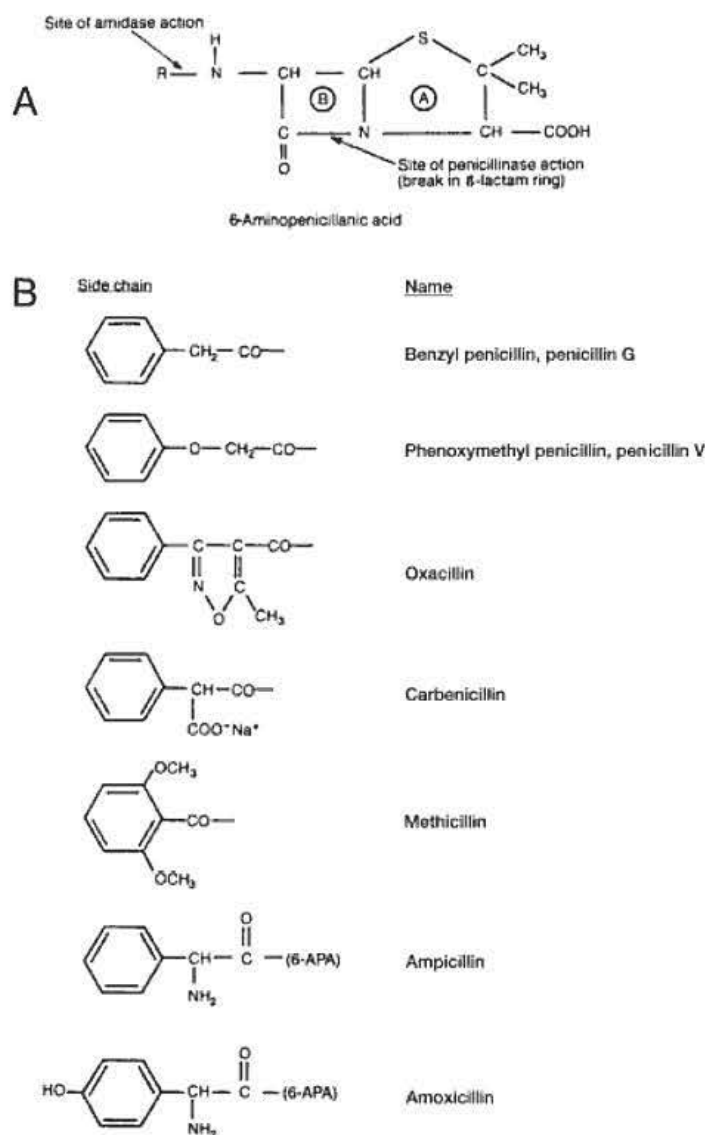


Figure 7.5. Structural formulae of some penicillins: (A) basic structure of penicillin G; (B) structures that can be substituted at the R to produce a new penicillin.

reus beta-lactamase (penicillinase). Subsequently, orally administered penicillins with a broader spectrum of activity, which involved greater Gram-negative antibacterial activity, and penicillins active against *P. aeruginosa* were developed. Despite considerable effort at identifying beta-lactamase resistant penam penicillins, however, with the exception of temocillin, extended spectrum penicillins are ineffective against beta-lactamase producing Gram-negative bacteria. For this reason, the use of penicillins against common Gram-negative bacteria is limited in favor of more recently introduced cephalosporin beta-lactams or combination with beta-lactamase inhibitors (Chapter 8).

Mechanism of Action

The targets of all beta-lactam drugs are the penicillin binding proteins (PBPs) found on the outside of the cytoplasmic membrane, which are involved in synthesizing and remodelling the cell wall. Susceptibility of a bacterium to a penicillin depends on a combination of affinity for the PBP, ability to penetrate the cell wall, and ability to resist beta-lactamase enzymes (Figure 7.3, 7.4). There are usually four to seven PBPs present in bacteria that are the targets for penicillins. The bactericidal effect in Gram-negative bacteria results from osmotically-induced lysis of cells weakened by loss of their peptidoglycan layer, although the exact mechanism is unknown. In Gram-positive bacteria, which

Table 7.1. Classification of the six groups of penam penicillins (6-aminopenicillanic acid derivatives).

Group	Important derivatives	Antimicrobial advantage
1. Benzyl penicillins	Procaine, benzathine (long acting forms)	Gram-positive bacteria
2. Orally absorbed benzyl penicillins	Phenoxymethyl penicillin	Gram-positive bacteria
3. Antistaphylococcal isoxazoyl penicillins	Cloxacillin, dicloxacillin, oxacillin, methicillin, nafcillin	Activity against <i>S. aureus</i>
4. Extended (broad) spectrum penicillins	Aminobenzylpenicillins (ampicillin, hetacillin, pivampicillin, amoxicillin); amidopenicillins (mecillinam)	Broader spectrum than benzyl penicillins, but beta-lactamase sensitive
5. Antipseudomonal penicillins	Ureidopenicillins (azlocillin, mezlocillin, piperacillin); carboxypenicillins (carbenicillin, ticarcillin)	<i>P. aeruginosa</i> activity, reduced Gram-positive
6. Beta-lactamase resistant penicillins	Temocillin	Beta-lactamase resistance

Table 7.2. Activity ($\mu\text{g/ml}$) of penicillins against bacteria of human origin (usual MIC).

Organisms	Narrow-spectrum penicillins		Penicillinase-stable penicillins		Broad-spectrum penicillins	
	Penicillin G	Penicillin V	Methicillin	Cloxacillin	Ampicillin	Carbenicillin
<i>Staphylococcus aureus</i>						
beta-lactamase -	0.02	0.05	1.25	0.1	0.05	1.25
Beta-lactamase +	R	R	2.5	0.25	R	25
<i>Streptococcus agalactiae</i>	0.005	0.01	0.2	0.06	0.02	0.2
Beta-hemolytic streptococci	0.005	0.01	0.2	0.04	0.02	0.2
<i>S. faecalis</i>	3	6	R	R	1.5	50
<i>Clostridium perfringens</i>	0.05	0.1	1	0.5	0.05	0.5
<i>Escherichia coli</i>	50	125	R	R	5	5
<i>Proteus mirabilis</i>	5	50	250	R	1.25	2.5
<i>Proteus</i> , indole +	R	R	R	R	250	5
<i>Klebsiella pneumoniae</i>	250	R	R	R	250	250
<i>Enterobacter</i> spp.	R	R	R	R	250	12.5
<i>Pseudomonas aeruginosa</i>	R	R	R	R	R	50

R, Resistant.

Source: Garrod et al. (1981) with permission.

have considerably greater quantities of peptidoglycan in their cell wall than Gram-negative bacteria, beta-lactams prevent the final peptidoglycan cross-linking that gives peptidoglycan its strength. Release of lipoteichoic acid causes a suicide response via degradation of peptidoglycan by autolysins (endogenous endopeptidase, carboxypeptidase PBPs).

For some Gram-positive cocci, exposure to beta-lactam antibiotics above an optimal killing concentration results in a reduction of killing, which can be considerable (the "Eagle" or paradoxical effect). Its basis appears to be interference of growth by penicillin binding to PBPs other than the major target PBP. Since beta-lactams are effective only against growing, ac-

tively cell-wall synthesizing bacteria, failure to grow results in failure to be killed. The Eagle effect is an important concept, since there may be a tendency to overdose with beta-lactam antibiotics because they are generally so safe.

Antimicrobial Activity

Benzyl penicillin and orally administered benzyl penicillins (phenoxymethyl penicillin) have outstanding activity against many Gram-positive bacteria, notably beta-hemolytic streptococci, non-resistant staphylococci, *Actinomyces* spp., *Bacillus* spp., *Clostridium* spp., *Corynebacterium* spp., and *Erysipelothrix rhusiopathiae*. Susceptible Gram-negative species include

some *Bacteroides* spp., some *Fusobacterium* spp., and a variety of Gram-negative aerobic bacteria such as *Haemophilus* spp., and many *Pasteurella* spp. (Table 7.2). Enterobacteriaceae, *Bacteroides fragilis*, most *Campylobacter* spp., *Nocardia* spp. and *Pseudomonas* spp. are resistant. Penicillinase resistant, antistaphylococcal isoxazolyl penicillins (cloxacillin, dicloxacillin, methicillin, nafcillin, oxacillin) have activity similar to but slightly less than that of benzyl penicillin, with the exception that they are active against penicillinase-producing *S. aureus* (Table 7.2). Extended spectrum penicillins (aminobenzylpenicillins such as ampicillin and its esters, and amoxicillin) retain the activity of benzyl penicillin against Gram-positive bacteria but have increased activity against Gram-negative bacteria including *E. coli*, *Proteus* spp., and *Salmonella* spp. They are ineffective against *P. aeruginosa* and are inactivated by beta-lactamases. Mecillinam, another member of the extended penicillin group, differs from aminobenzylpenicillins in its lower activity against Gram-positive bacteria but considerably greater activity against Gram-negative bacteria including a broad-spectrum of the Enterobacteriaceae, although it is still inactivated by many beta-lactamases. Penicillins (carboxypenicillins, ureidopenicillins) active against *P. aeruginosa* (carbenicillin, azlocillin, mezlocillin, piperacillin) are effective against both Gram-positive and Gram-negative bacteria, including *P. aeruginosa* (Table 7.2).

Resistance to Penam Penicillins

Most resistance results from production of beta-lactamase enzymes. Modification of PBPs with reduced drug affinity or reduced bacterial permeability are additional and sometimes concurrent mechanisms of intrinsic or acquired resistance to penam penicillins. Efflux mechanisms have also been recognized recently. Beta-lactamases are discussed in Chapter 8. Resistance because of exogenously-produced beta-lactamase is now widespread in *S. aureus*, particularly in clinical isolates, as a result of bacteriophage- or plasmid-mediated resistance. Among Gram-negative bacteria, plasmids encoding beta-lactamases have also become widespread and are the cause of extensive acquired resistance. Modification of PBPs is increasingly important as another mechanism of resistance to penam penicillins.

The most important type of penam penicillin resistance in human medicine is methicillin (oxacillin)-

resistance in *S. aureus* (MRSA), which is widespread in humans in some countries, notably Japan and the United States. Resistance because of this mechanism has emerged in animals in recent years, notably in dogs and horses, and reflects the incidence of infection in humans from whom these strains were acquired (Rich and Roberts, 2004; Weese, 2005; Weese, et al. 2005). In addition, animal MRSA strains are often hospital-associated and can contaminate veterinary hospital environments to a remarkable extent (Seguin et al., 1999; Weese et al., 2004). Human subclinical and even clinical infections have been acquired from animal sources (Weese et al., 2005). MRSA are regarded as resistant to all beta-lactam antimicrobials and are commonly but not always resistant to other antimicrobials. Methicillin resistance is more frequent in coagulase-negative *Staphylococcus* spp., which may rarely be significant as nosocomial infections in hospitalized animals.

Pharmacokinetic Properties

The penicillins are organic acids that are available as the sodium or potassium salts of the free acid. In dry, crystalline form, penicillins are stable but lose their activity rapidly when dissolved. Apart from the isoxazolyl penicillins (cloxacillin, dicloxacillin, oxacillin) and penicillin V, acid hydrolysis limits the systemic availability of most penicillins from oral preparations. The penicillins (pK_a 2.7) are predominantly ionized in plasma, have relatively small apparent volumes of distribution (0.2–0.3 L/kg), and have short half-lives (0.5–1.2 hours) in all species of domestic animals. After absorption, they are widely distributed in the extracellular fluids of the body, but cross biologic membranes poorly because they are ionized and poorly lipid soluble. Concentration in milk, for example, is about one-fifth that of serum. Entry across biologic membranes or through the blood-brain or blood-cerebrospinal fluid barrier is enhanced by inflammation, so that inhibitory drug concentrations may be attained at these sites that are normally inaccessible to penicillin.

Penicillins are eliminated almost entirely by the kidneys, which results in very high levels in the urine; nafcillin is an exception, as it is excreted mainly in bile. Renal excretion mechanisms include glomerular filtration and active tubular secretion. The latter is subject to competitive inhibition by other organic acids such as probenecid. Impaired renal function delays excretion

of the penicillins, but the wide margin of safety of this class of drug offsets the absolute need to adjust dosage.

Drug Interactions

Penicillins are usually synergistic with the aminoglycosides against many bacteria, which are normally resistant to each drug alone. Cell wall disruption from the penicillin enhances penetration of the aminoglycoside. Such synergism may occur with penicillinase-producing *S. aureus*. Penicillins are synergistic against these organisms with drugs that bind beta-lactamase enzymes, such as cloxacillin, clavulanic acid, sulbactam, tazobactam and some cephalosporins. Aminobenzylpenicillins and ureidopenicillins are increasingly combined with beta-lactamase inhibitors (Chapter 9).

Toxicity and Adverse Effects

Penicillins and beta-lactam antibiotics are remarkably free of toxic effects even at doses grossly in excess of those recommended. The major adverse effects are acute anaphylaxis and collapse; milder hypersensitivity reactions (urticaria, fever, angioneurotic edema) are more common. All penicillins are cross-sensitizing and cross-reacting, but cross-reactions occur in only about 5-8% of human patients treated with cephalosporins. Anaphylactic reactions are less common after oral than parenteral administration. Penicillins must not be used in animals known to be sensitive. Less common adverse reactions include hemolytic anemia and thrombocytopenia.

Dosage Considerations

Beta-lactams lyse and kill bacteria at concentrations above the MIC. Post-antibiotic effects are observed only for staphylococci in vivo, so that dosage requires drug concentrations exceeding the MIC for most of the dosage interval. Excessive drug concentrations may be counterproductive because of the Eagle effect described earlier, where dramatic reduction of killing occurs in the presence of supraMIC concentrations of the beta-lactam antibiotic.

Clinical Usage

Penicillins (Table 7.1) are important antibacterial drugs in the treatment of infections in animals. The often exquisite susceptibility of Gram-positive bacteria, such as the beta-hemolytic streptococci, means that benzyl penicillin is a drug of choice for these infections. Antistaphylococcal penicillins are widely

used in the prevention and treatment of staphylococcal infections in dairy cows. The extended spectrum penicillins, particularly aminobenzylpenicillins, have lost much of their potency against Gram-negative bacteria over the decades, but have been revitalized by their combination with beta-lactamase inhibitors (Chapter 9). The antipseudomonal penicillins remain important for this activity but are rivaled by antipseudomonal cephalosporins.

Group 1: Benzyl Penicillin and Long-Acting Parenteral Forms

Sodium benzyl penicillin G is available as the benzyl, the procaine benzyl, and the tribenzyl ethylenediamine (benzathine) forms. Frequent dosing of benzyl penicillin is required due to its rapid excretion, so that long acting/delayed absorption forms (procaine, benzathine) have been developed, with procaine penicillin being the most extensively used because dosing frequency is usually every 24 hours. The principle behind the use of procaine and benzathine penicillin is that both forms delay absorption from the injection site. Thus, while the elimination half-life is the same, the absorption half-life is much longer, reducing the need for frequent dosing. Delayed absorption also means a lower peak concentration and increases the risk of violative penicillin residues in food animals.

Antimicrobial Activity

The activity of penicillin G was originally defined in units. Crystalline sodium penicillin G contains approximately 1,600 units/mg (1 unit = 0.6 µg; 1 million units of penicillin = 600 mg or 0.6 g). Most semisynthetic penicillins are prescribed by weight (mg/kg) rather than units.

Good susceptibility ($MIC \leq 0.12 \mu\text{g/ml}$) is shown by many aerobic Gram-positive bacteria including all beta-hemolytic streptococci (such as *Streptococcus agalactiae*, *S. canis*, *S. zooepidemicus*, *S. dysgalactiae*, *S. suis*, *S. uberis*), *Bacillus anthracis*, *Actinomyces* spp., most corynebacteria (including *C. pseudotuberculosis*, *C. renale*), *Erysipelothrix rhusiopathiae*, and most *Listeria monocytogenes* (Table 7.2). Susceptible anaerobes include *Clostridium* spp., most *Fusobacterium* spp., and some *Bacteroides*. Susceptible Gram-negative aerobes include *Histophilus somni*.

Variable susceptibility is shown by *S. aureus* and other staphylococci, although in the absence of resistance, staphylococci are highly susceptible.

Moderate susceptibility (MIC 0.25–2 µg/ml) (which may sometimes vary because of acquired resistance), is shown by *Actinobacillus* spp., *Borrelia* spp., *Brucella* spp., *Haemophilus* spp., *Leptospira* spp., *Moraxella* spp., *Pasteurella* spp., *Proteus* spp., *Taylorella equigenitalis*, and *Brachyspira* spp.

Resistance (MIC ≥ 4 µg/ml) is shown by Enterobacteriaceae (other than some *Proteus* spp.), *Bacteroides fragilis*, *Bordetella* spp., most *Campylobacter* spp., and *Nocardia* spp.

Antibiotic Resistance

Despite extensive use of penicillin in veterinary medicine for many years, most Gram-positive bacteria remain susceptible to the drug. *Staphylococcus aureus* is an exception. The beta-lactamase enzymes of *S. aureus* are mainly active against penicillin G, ampicillin, and carbenicillin but hydrolyze penicillinase-stable penicillins (methicillin, cloxacillin) and cephalosporins poorly. Methicillin-resistant *S. aureus* (MRSA) are increasingly emerging in animals from their reservoir in humans, and may become increasingly problematic. Resistance in usually susceptible Gram-negative bacteria such as *Haemophilus* and *Pasteurella* is the result of R plasmid-mediated production of beta-lactamases.

Pharmacokinetic Properties

These were discussed earlier under general properties of penam penicillins. Acid hydrolysis in the stomach limits the systemic availability of benzyl penicillin administered orally.

Drug Interactions

Penicillin G is synergistic with the aminoglycosides against many Gram-positive bacteria, except those showing high-level aminoglycoside resistance. Such synergism may be seen even with penicillinase-producing *S. aureus*. Penicillin is synergistic against these organisms with drugs that bind beta-lactamase enzymes (Chapter 9). Penicillin G has been combined with streptomycin for use in animals, but there is little clinical evidence supporting the clinical value of the combination (Whittem and Hanlon 1997). For this reason, and more particularly because streptomycin is

associated with tissue residues, the combination is no longer available in some countries. In addition, there are significant differences in pharmacokinetic properties between different combined preparations.

Toxicities and Adverse Effects

The parent benzyl penicillin and its numerous derivatives are relatively safe drugs; toxic effects were described under General Considerations. Many of the acute toxicities reported in animals are the result of the toxic effects of the potassium or procaine with which penicillin is combined in the dosage form. To avoid cardiac arrest, care should be taken with the rate at which potassium penicillin G is injected IV; administration of the sodium salt is safer. Procaine penicillin G should never be given by this route. In high doses given IM, the procaine form may cause nervous excitement (incoordination, ataxia, excitability) and death, particularly in horses. It should not be administered to horses within 2 weeks before a competition to avoid procaine-positive drug test results. Procaine penicillin should be stored in the refrigerator and not used past expiration dates; repeated use of the same injection site should be avoided, especially in horses. Severe, immune-mediated hemolytic anemia with icterus has been reported in horses.

Administration and Dosage

Recommended dosages are shown in Table 7.3.

Because of the relative safety of penicillins, their dosage can be tailored to the susceptibility of the infecting bacteria more than with any other class of antibiotic. The effectiveness of penicillin therapy is related to the time that plasma or tissue concentration exceeds the MIC of the pathogen. Because of the short half-lives of penicillins, preparations that provide rapid absorption must be administered frequently (every 6 hours). Low systemic availability from oral forms must be offset by increasing the size of the dose.

Penicillin G is available as a potassium or sodium salt that can be administered parenterally as a freshly prepared solution. Procaine penicillin G is a special form developed to prolong absorption from the IM injection site. A single dose of 25,000 units/kg provides effective serum concentrations against susceptible bacteria for at least 12 hours and generally for up to 24 hours in all species of domestic animals. For moderately susceptible bacteria, high doses of procaine penicillin given once daily may be useful; an example is ad-

Table 7.3. Usual dosages of penam penicillins in animals.

Drug	Route	Dose (IU or mg/kg)	Interval (hr)	Comments
Penicillin G, sodium aqueous	IM, IV	15-20,000 IU	6-8	
Procaine penicillin G	IM	25,000 IU	24	Every 12 hours for serious infections
Benzathine penicillin	IM	40,000 IU	72	Highly susceptible bacteria only; little use
Penicillin V	Oral	10	6-8	Erratic absorption; amoxicillin preferred
Cloxacillin, dicloxacillin, methicillin, oxacillin	Oral	15-25	6-8	Monogastrates only; avoid ingesta
Ampicillin sodium	IM, IV	10-20	6-8	
Ampicillin (Hetacillin)	Oral	10-20	8	Monogastrates only; avoid ingesta
Amoxicillin	Oral	10-20	8-12	Monogastrates only
Amoxicillin	IM (SC)	10	12	
Amoxicillin, long-acting	IM	15	48	Very susceptible bacteria only
Amoxicillin trihydrate	IM	10-20	12	
Pivampicillin	Oral	25	12	Monogastrates only
Carbenicillin, indanyl sodium	Oral	33	6-8	Urinary tract only
Carbenicillin	IM, IV	33	6-8	
Ticarcillin	IV (IM, SC)	25-40	8	Often used with clavulanic acid
Piperacillin	IV (IM)	50	8	May be used with tazobactam

ministration of 45,000 units/kg in the once-daily treatment of bovine *Mannheimia haemolytica* pneumonia. More clinical data is needed on such high dosing, since the Eagle effect may reduce the efficacy of the drug. Oral potassium penicillin G has been used to treat canine urinary tract infections caused by *E. coli* or *Proteus mirabilis*. The response is due to the high concentrations of penicillin that are attained in urine.

Benzathine penicillin is a long-acting, slow-release formulation of penicillin G administered every 72 hours. Serum concentrations are usually so low that it can only be recommended for extremely susceptible bacteria. It is a frequent cause of residue violations in food animals.

Clinical Applications

The general clinical applications of penicillin G are shown in Table 7.4.

Penicillin G is the drug of choice in treating infections caused by Gram-positive bacteria such as streptococci, corynebacteria, *Erysipelothrix*, clostridia, and perhaps of *Listeria*, and some Gram-negative bacteria such as *H. somni*, *Pasteurella*, and many anaerobes. In addition, it is a drug of choice in treating the spirochetal agent of Lyme disease, *Borrelia burgdorferi*. The advantages of penicillin G are its potent bactericidal activity against susceptible bacteria and its wide margin of safety; dosage can be tailored to the susceptibility of the pathogen by selecting the form of drug to be

administered. Disadvantages are its activity only against actively growing bacteria, its narrow spectrum, widespread resistance in *S. aureus* and Gram-negative bacteria, and the drug's failure to cross biological membranes well, except in acute inflammation.

Cattle, Sheep, and Goats

Penicillin G is the most commonly used antibiotic for food animals. It was initially licensed at an inappropriately low dosage; more recent approvals and withdrawal times are based on appropriate pharmacokinetics/pharmacodynamics. Parenterally administered penicillin G is the drug of choice for the treatment of disease caused by susceptible bacteria including anthrax, clostridial infections, *Corynebacterium renale* infection, *H. somni* infection, pneumonic pasteurellosis caused by sensitive *Mannheimia* and *Pasteurella*, and infections caused by nonsporeforming anaerobes such as *Fusobacterium necrophorum* and *Porphyromonas asaccharolytica*. Penicillin G's poor activity against slowly multiplying bacteria and relative inability to penetrate biologic membranes may explain its often disappointing effect in treating *A. pyogenes*, actinomycosis, and chronic *S. aureus* mastitis. For most conditions that are responsive to penicillin, a dosage of 20–25,000 IU/kg once daily is adequate for procaine penicillin G.

Listeriosis has been successfully treated with a daily dose of 44,000 units/kg of procaine penicillin admin-

Table 7.4. Applications of penicillin G (or penicillin V) in clinical infections in animals.

Species	Primary applications	Secondary applications
Cattle, sheep, goats	Anthrax, clostridial and corynebacterial infections, <i>A. pyogenes</i> , streptococcal mastitis, listeriosis	Actinobacillosis, anaerobic infections, possibly infectious keratoconjunctivitis, leptospirosis
Swine	Streptococcal, clostridial infections, erysipelas, <i>A. pyogenes</i> , <i>A. suis</i>	Glasser's disease, pasteurellosis, anaerobic infections
Horses	Streptococcal and clostridial infections	Actinobacillosis, anaerobic infections
Dogs, cats	Streptococcal and clostridial infections	Cat bite abscess, anaerobic infections, leptospirosis

istered for 7–14 days, but ampicillin is preferred. Penicillin G is effective for acute leptospirosis, although again, ampicillin is probably preferable. Procaine penicillin G (300,000–600,000 units in 1–2 ml) administered subconjunctivally has been used extensively in the treatment of *Moraxella bovis* keratoconjunctivitis, since this maintains therapeutic concentrations for up to 36 hours. One controlled study questioned the value of this treatment (Allen et al., 1996).

Pneumonic pasteurellosis has been treated successfully with daily intramuscular or subcutaneous injections of 45,000 units/kg of procaine penicillin. Resistance among *M. haemolytica*, however, is increasing and further increases in dose are not justified. Serious, acute mastitis caused by streptococci or susceptible *S. aureus* can be treated by IM procaine penicillin 20–25,000 IU/kg, q12 or q24 hours depending on severity, as a probably useful adjunct to frequent stripping of the infected quarter. Penicillin is more commonly administered intramammarily, often combined with streptomycin, and has given excellent results in the treatment of streptococcal infections during lactation, but only modest results against *S. aureus*. Intramammary treatment of susceptible Gram-positive cocci with procaine penicillin G and neomycin showed no advantage over procaine penicillin G alone (Taponen et al., 2003). Penicillin G in fixed combination with streptomycin has been used successfully against severe dermatophilus infection but this combination is no longer available in many countries.

Swine

Penicillin is the parenteral drug of choice in preventing and treating streptococcal, clostridial, corynebacterial, and erysipelas infections. For acute erysipelas and streptococcal infections, procaine penicillin is preferred, but benzathine penicillin is sometimes used in

prophylaxis. *Streptococcus suis* meningitis may be treated successfully with daily injections of procaine penicillin given early in the disease. Attempts to eradicate *S. suis* infection by treating farrowing sows with benzathine penicillin have yielded variable results. Oral administration of procaine penicillin significantly reduced the incidence of spontaneous *S. suis* meningitis in pigs, a surprising effect in view of the low tissue concentrations achieved (McKellar et al., 1987). Penicillin-streptomycin combination (25 mg/kg) administered for 1, 3 or 5 days removed the kidney carrier state in swine infected with *Leptospira pomona* (Allt and Bolin, 1996).

Horses

Penicillin G is used against beta-hemolytic streptococci; in neonatal foals for *S. zooepidemicus* polyarthritis and meningitis; and in adult animals for infections of wounds, lower respiratory and urinary tracts, and the uterus, where it may be given by parenteral administration and local infusion. It is the drug of choice in strangles caused by *Streptococcus equi*, when treatment is required. Penicillin is the preferred antibiotic to treat tetanus. Injection of procaine penicillin G in the neck or biceps gives higher serum concentrations than injection in the gluteal muscle or SC (Firth et al., 1986). Penicillin should not be administered orally to horses because of its poor absorption and tendency to disturb digestion.

Dogs and Cats

Penicillin G is a drug of choice for streptococcal and clostridial infections, for actinomycosis, and for infections caused by susceptible Gram-negative bacteria such as *P. multocida*. Because of penicillin G's activity against anaerobic bacteria, it is particularly suitable in the treatment of periodontal disease, tooth abscesses, wound infections, and perhaps pyometra. However,

amoxicillin (and to a lesser extent ampicillin) is preferred for all these uses. Unlike penicillin G, which is erratically absorbed in dogs and cats after oral administration and which therefore is administered parenterally, amoxicillin is well absorbed following oral administration, which increases tissue concentrations and decreases the amount of drug remaining in the gut to cause intestinal disturbance. Because of the very high urinary concentrations attained after administration of penicillin G and amoxicillin by any route, either drug may be used in the treatment of canine urinary tract infections caused by *S. aureus* (even penicillinase-producing), streptococci, *E. coli*, and *P. mirabilis*.

Poultry

Oral penicillin is used in the prevention and treatment of necrotic enteritis, ulcerative enteritis, and intestinal spirochetosis and, in combination with streptomycin, in treating erysipelas in turkeys.

Bibliography

- Allt DP, Bolin CA. 1996. Preliminary evaluation of antimicrobial agents for treatment of *Leptospira interrogans* serovar pomona infection in hamsters and swine. *Am J Vet Res* 57:59.
- Allen LJ, et al. 1995. Effect of penicillin or penicillin and dexamethasone in cattle with infectious bovine keratoconjunctivitis. *J Am Vet Med Assoc* 206:1200.
- Divers TJ. 1996. Penicillin therapy in bovine practice. *Comp Cont Ed Pract Vet* 18:703.
- Firth EC, et al. 1986. Effect of the injection site on the pharmacokinetics of procaine penicillin in horses. *Am J Vet Res* 47:2380.
- Garrod LP, et al. 1981. Antibiotics and chemotherapy, 5th ed. New York: Churchill Livingstone.
- McKellar QA, et al. 1987. Penicillin therapy of spontaneous streptococcal meningitis in pigs. *Vet Rec* 121:347.
- Papich MG, et al. 1994. Disposition of penicillin G after administration of benzathine penicillin G, or a combination of benzathine penicillin G and procaine penicillin. *Am J Vet Res* 55:825.
- Rich M, Roberts L. 2004. Methicillin-resistant *Staphylococcus aureus* isolates from companion animals. *Vet Rec* 154:310.
- Taponen S, et al. 2003. Efficacy of intramammary treatment with procaine penicillin G vs procaine penicillin G plus neomycin in bovine clinical mastitis caused by penicillin-susceptible, gram-positive bacteria—a double blind field study. *J Vet Pharm Therap* 26:193.
- Watson ADJ, et al. 1987. Systemic availability of penicillin V from six oral preparations in dogs. *J Vet Pharm Ther* 10:180.
- Weese JS. 2005. Methicillin-resistant *Staphylococcus aureus*: an emerging pathogen in small animals. *J Am Vet Hosp Assoc* 41:150.
- Weese JS, et al. 2004. Isolation of methicillin-resistant *Staphylococcus aureus* from the environment in a veterinary teaching hospital. *J Vet Intern Med* 18:468.
- Weese JS, et al. 2005. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel, 2000-2002. *Emerg Infect Dis* 11:430.
- Whittem T, Hanlon D. 1997. Dihydrostreptomycin or streptomycin in combination with penicillin G in dairy cattle therapeutics: A review and re-analysis of published data. Part 1: Clinical pharmacology. *NZ Vet J* 45:178.

Group 2: Orally Absorbed Penicillins

Phenoxymethyl penicillin (penicillin V) resists stomach acid hydrolysis and is therefore administered orally. It has a spectrum of activity similar to benzyl penicillin, and is therefore used for the same purposes in monogastrates. Oral administration of penicillin V is used in the effective prophylaxis and treatment of *S. suis* meningitis in swine.

Group 3: Antistaphylococcal Isoxazolyl Penicillins: Cloxacillin, Dicloxacillin, Methicillin, Nafcillin and Oxacillin

The antistaphylococcal penicillins are resistant to *S. aureus* penicillinase and are used mainly in the treatment or prevention of bovine staphylococcal mastitis. The isoxazolyl penicillins (cloxacillin, oxacillin) are acid stable and may be given orally to monogastric animals, for example, in the treatment of staphylococcal skin infections in dogs. Penicillinase production in *S. aureus* may be detected by the use of nitrocefin-impregnated paper disks.

All are resistant to *S. aureus* penicillinase, although activity against other penicillin-sensitive bacteria is less than that of penicillin G. Activity of the different drugs is similar in vivo.

Reports of Methicillin-resistant *S. aureus* (MRSA) are increasing, particularly in dogs and horses that are or have been in veterinary hospitals. Many of these strains are of human origin. Resistance to methicillin in bovine *S. aureus* isolates is rare. Figures purporting to show extensive resistance in bovine isolates probably reflect inappropriate test conditions or drug inactivity, as methicillin deteriorates readily in storage.

Methicillin resistant (heteroresistant) *S. aureus* may be overlooked. While no single method is ideal, methicillin resistant *S. aureus* are best detected using oxacillin disks, with *S. aureus* grown 18–24 hours at 30°C or 35°C. Increasingly, laboratories will also use PCR to identify the *mecA* gene. Heteroresistant *S. aureus* are often multidrug-resistant (other beta-lactams, aminoglycosides, macrolides, tetracyclines) but susceptible to rifampin, fluoroquinolones, and trimethoprim-sulfamethoxazole.

Activity of antistaphylococcal isoxazoyl penicillins against streptococci causing mastitis in cows is good. Cure rates approximate those for penicillin-streptomycin combinations. While apparent clinical cure of *S. aureus* mastitis is usual, bacteriologic cure is often disappointing. Daigneault and George (1990) established the value of high topical doses (375 mg) of benzathine cloxacillin applied twice at 3-day intervals in the treatment of experimental *M. bovis* keratoconjunctivitis.

In dogs, IV use of nafcillin during surgery to prevent staphylococcal infection has been associated with the development of acute renal failure within 2–4 days of surgery, probably as a result of direct renal damage by the drug (Pascoe et al., 1996). Studies of the pharmacokinetics of dicloxacillin in dogs suggest that IM administration (25 mg/kg, q8 hours) is more reliable than oral administration in achieving serum concentrations of drug consistently \leq MIC of penicillinase-producing *S. aureus* (Dimitrova et al., 1996).

Bibliography

- Daigneault J, George LW. 1990. Topically applied cloxacillin for treatment of experimentally induced bovine keratoconjunctivitis. *Am J Vet Res* 51:376.
- Dimitrova DJ. 1996. Pharmacokinetics of dicloxacillin sodium following intravenous and intramuscular administration to domestic cats. *J Vet Pharm Ther* 19:405.
- Dimitrova DJ, et al. 1998. Dicloxacillin pharmacokinetics in dogs after intravenous, intramuscular and oral administration. *J Vet Pharm Ther* 21:414.
- Pascoe PJ, et al. 1996. Case-control study of the association between intraoperative administration of nafcillin and acute postoperative development of azotemia. *J Am Vet Med Assoc* 208:1043.
- Rich M, Roberts L. 2004. Methicillin-resistant *Staphylococcus aureus* isolates from companion animals. *Vet Rec* 154:310.
- Weese JS, et al. 2005. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel, 2000–2002. *Emerg Infect Dis* 11:430.

Group 4: Extended-spectrum Penicillins. Aminobenzyl Penicillins: Ampicillin, Amoxicillin

Ampicillin, amoxicillin, and the related esters bacampicillin, hetacillin, pivampicillin and talampicillin have similar antimicrobial activity, but amoxicillin and possibly pivampicillin have the advantage of achieving higher tissue concentrations because of better absorption from the intestine. The broad-spectrum aminobenzylpenicillins are slightly less active than penicillin G against Gram-positive and anaerobic bacteria and are susceptible to staphylococcal penicillinase. These broad-spectrum drugs, however, have considerably greater activity against Gram-negative bacteria such as *E. coli*, *P. mirabilis*, and *Salmonella*. Nevertheless, acquired resistance has considerably reduced the effectiveness of these drugs. An exciting development has been their combination with beta-lactamase-inhibiting drugs, which increases their effectiveness considerably (Chapter 9), and with which these drugs should generally be combined.

Antimicrobial Activity

Good susceptibility (MIC \leq 1). As for benzyl penicillin group but includes *Borrelia* spp. and *Leptospira* spp., which are highly susceptible; *Actinobacillus* spp., *Haemophilus* spp., *Moraxella* spp., *Pasteurella* spp. (Tables 7.2, 7.5).

Moderate susceptibility (MIC 2–4 μ g/ml). As for benzyl penicillin but also *Campylobacter* spp., enterococci, *R. equi*. Variable moderate activity (because of acquired resistance) against *E. coli*, *P. mirabilis*, and *Salmonella*. Acquired resistance in Enterobacteriaceae is widespread.

Resistance (MIC > 4 μ g/ml, approximately). *Bacteroides fragilis*, *B. bronchiseptica*, *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., other *Proteus* spp., *P. aeruginosa*, *Serratia* spp., *Y. enterocolitica*.

Antimicrobial Resistance

Plasmid- or integron-mediated, acquired resistance is common in Gram-negative bacteria and is often multiple, especially in most enterotoxigenic *E. coli* and *S. typhimurium*. Many *E. coli* that cause bovine mastitis are resistant. Aminobenzylpenicillins are susceptible to *S. aureus* beta-lactamase (Tables 7.2, 7.5).

Table 7.5. In vitro activity of extended-spectrum and antipseudomonal penicillins against various medically important opportunistic bacteria.

Organisms	Ampicillin		Mecillinam		Ticarcillin		Azlocillin		Piperacillin	
	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀
<i>Streptococcus agalactiae</i>	0.06	0.12	2	8	2	4	0.25	1	0.25	1
<i>Escherichia coli</i>	4	128	1	4	16	128	8	128	8	128
<i>Klebsiella pneumoniae</i>	128	128	2	128	128	128	32	128	8	128
<i>Citrobacter diversus</i>	4	128	0.5	4	16	128	4	8	4	4
<i>Enterobacter cloacae</i>	128	128	2	32	8	128	4	32	4	32
<i>Proteus mirabilis</i>	1	4	4	16	0.5	16	0.5	16	0.5	16
<i>Pseudomonas aeruginosa</i>	128	128	128	128	16	128	4	128	4	128
<i>Bacteroides</i> spp.*	1	32	2	16	4	32	2	8	2	4

*Other than *B. fragilis*.

Source: Modified from Prince and Neu (1983) with permission.

Pharmacokinetic Properties

The basic pharmacokinetic properties of penicillins were described under General Considerations. Both ampicillin and amoxicillin are relatively stable in acid. In dogs, the systemic availability of amoxicillin (60–70%) is about twice that of ampicillin (20–40%), so that peak blood concentrations are often twice or more those that occur after the same dose of ampicillin. The absorption of amoxicillin is unaffected by feeding, unlike ampicillin. Hetacillin and pivampicillin are esters of ampicillin developed to increase systemic availability, but it is questionable whether this occurs in dogs. Pivampicillin has significantly better bioavailability in horses than amoxicillin after oral administration (Ensink et al., 1992). Ampicillin is available as a sodium salt that can be administered parenterally in a freshly prepared solution. The trihydrate salts are less soluble and therefore poorly absorbed from the intestine, but form aqueous suspensions that can be injected IM or SC. These preparations produce low peak concentrations in the serum, but they extend the dosing interval to 12 hours. Long-acting preparations of ampicillin trihydrate, which produce therapeutic serum concentrations for 48 hours, have been introduced. The lower peak plasma concentrations, however, may decrease penetration of the antibiotic to sites of infection.

Drug Interactions

Aminobenzylpenicillins are commonly synergistic with aminoglycosides against Gram-positive bacteria and often also against Gram-negative bacteria, but

only if the latter are not resistant to both drugs. The broad-spectrum beta-lactamase inhibitors, clavulanic acid and sulbactam, show remarkable synergism with aminobenzylpenicillins against beta-lactamase-producing bacteria (Chapter 9).

Toxicities and Adverse Effects

Toxic effects are similar to those described under General Considerations. One hazard with broad-spectrum penicillins is the potential to disturb the normal intestinal flora. In dogs and cats, the effect may be less marked with amoxicillin, which is better absorbed. Ampicillin should not be administered to small rodents (guinea pigs, hamsters, gerbils) or to rabbits since it may produce clostridial colitis (*C. difficile* or in rabbits, *C. spiroforme*). Pivampicillin was associated with less diarrhea or loose feces in horses than observed with trimethoprim-sulfadiazine (Ensink et al. 1996a). Moderate diarrhea has been described in calves after several days' treatment with oral ampicillin, which appears to result from malabsorption caused by a direct effect on intestinal mucosa.

Administration and Dosage

Recommended dosages are shown in Table 7.3. The soluble sodium salts can be administered parenterally and orally, but the poorly soluble trihydrate form should only be administered IM. Once reconstituted, aqueous sodium salts are stable only a few hours. Because of their short half-lives, preparations that are rapidly absorbed should be administered every 6 hours to maintain serum drug concentrations over 1

µg/ml for a significant length of time. Amoxicillin is preferred over Ampicillin for oral administration because it is better absorbed than ampicillin, and its absorption is unaffected by feeding. Another advantage of oral amoxicillin over ampicillin is that it can be given twice daily to small animals. Long-acting preparations of amoxicillin are available, but it is doubtful whether they maintain therapeutic serum concentrations for the 48-hour recommended dosing interval.

Clinical Applications

The aminobenzylpenicillins are bactericidal, relatively nontoxic drugs with a broader spectrum of activity than penicillin G and are better distributed in the body. Even with these advantages, relatively high doses are required to treat infections caused by Gram-negative bacteria. The relatively high prevalence of acquired resistance has limited their place. Amoxicillin is the best penicillin for the treatment of urinary tract infections and enteric infections caused by susceptible organisms. It has similar activity to penicillin G in the treatment of anaerobic infections. Although amoxicillin offers pharmacokinetic advantages over ampicillin, it has some of the same difficulty as ampicillin in attaining sufficient concentrations in tissues to treat Gram-negative bacterial infections.

The main clinical applications are similar to those shown in Table 7.4. Amoxicillin is a drug of choice in the treatment of leptospirosis. Ampicillin is preferred to penicillin to treat listeriosis.

In cattle, sheep and goats, oral ampicillin has been used to treat *E. coli* and *Salmonella* infections, but acquired resistance limits their effectiveness for this purpose. Ampicillin is effective against bovine respiratory disease but seems to offer no advantage over penicillin G. Ampicillin has been used intramammarily in the treatment of coliform mastitis, but resistance largely limits its use. Long-acting amoxicillin administered twice at 15 mg/kg IM q48 hours was effective in removing the *Leptospira hardjo* kidney carrier state from the majority of experimentally infected cattle (Smith et al., 1997).

Indications in horses for ampicillin or amoxicillin are few since they offer little advantage over benzyl penicillins, largely because of acquired resistance in Gram-negative bacteria. One indication is in the treatment of *R. equi*, but drugs should be administered IM at 11–15 mg/kg every 6 hours. Oral administration of amoxicillin (or preferably pivampicillin) is appropri-

ate for infections in foals caused by organisms with good susceptibility but cannot be recommended for adult horses.

Ampicillin or amoxicillin is used in the treatment of canine urinary tract infections, because over 90% of *S. aureus*, streptococci, and *P. mirabilis*, nearly 90% of *E. coli*, and 65% of *Klebsiella* are regarded as susceptible to urinary concentrations of the drug. Nevertheless, treatment results in one study were not conspicuously better than those obtained with penicillin G. The combination of clavulanic acid-amoxicillin is preferred for these purposes. Clinical trials in cats showed once-daily dosing with a 50 mg tablet of amoxicillin to be as effective as twice-daily dosing. Field trial comparison in cats of 50 mg amoxicillin twice daily versus 50 mg hetacillin twice daily showed a significant advantage for amoxicillin (Keefe, 1978). Amoxicillin, metronidazole and omeprazole as a triple combination has been used to produce bacteriologic cure in the treatment of *Helicobacter* gastritis in cats, but the organism could still be detected by polymerase chain reaction (PCR) (Perkins et al., 1996). Triple therapy with amoxicillin, metronidazole, and bismuth subcitrate has been used to eradicate gastric *Helicobacter* from dogs, although there is still considerable uncertainty of the role of these bacteria in canine gastritis (Happonen et al., 2000). Unfortunately, PCR does not distinguish between viable and nonviable organisms. Amoxicillin produced clinical cure of *B. burgdorferi* infection in the majority of treated dogs, but the organism was not eradicated (Straubinger et al., 1997).

In poultry, ampicillin is sometimes administered orally for the prevention or treatment of *E. coli* or *S. aureus* septicemia, or of salmonellosis.

Bibliography

- Agerso H, et al. 1998. Water medication of a swine herd with amoxycillin. *J Vet Pharm Ther* 21:199.
- Baggot JD, et al. 1990. Oral dosage of penicillin V in adult horses and foals. *Equine Vet J* 22:290.
- Ensink JM, et al. 1996a. Side effects of oral antimicrobial agents in the horse: a comparison of pivampicillin and trimethoprim/sulphadiazine. *Vet Rec* 138:253.
- Ensink JM, et al. 1996b. Oral bioavailability and in vitro stability of pivampicillin, bacampicillin, talampicillin and ampicillin in horses. *Am J Vet Res* 57:1021.
- Errecalde JO, et al. 2001. Pharmacokinetics of amoxycillin in normal horses and horses with experimental arthritis. *J Vet Pharm Therap* 24:1.

- Happonen I, et al. 2000. Effect of triple therapy on eradication of canine gastric helicobacters and gastric disease. *J Small Anim Pract* 41:1.
- Keefe TJ. 1978. Clinical efficacy of amoxicillin oral suspension. *Feline Pract* 8:33.
- Kueng G, Wanner M. 1994. Bioavailability of different forms of amoxycillin administered orally to dogs. *Vet Rec* 135:552.
- Prince AS, Neu HC. 1983. New penicillins and their use in pediatric practice. *Pediatr Clin North Am* 32:3.
- Perkins SE, et al. 1996. Use of PCR and culture to detect *Helicobacter pylori* in naturally infected cats following triple antimicrobial therapy. *Antimicrob Agents Chemother* 40:1486.
- Smith CR, et al. 1997. Amoxycillin as an alternative to dihydrostreptomycin sulphate for treating cattle infected with *Leptospira borgpetersenii* serovar *hardjo*. *Aust Vet J* 75:818.
- Straubinger RK, et al. 1997. Persistence of *Borrelia burgdorferi* in experimentally infected dogs after antibiotic treatment. *J Clin Microbiol* 35:111.

Group 4: Extended-spectrum Penicillins. Amidopenicillins: Mecillinam

Mecillinam (amidinopenicillin) is active against a broader range of Enterobacteriaceae than ampicillin, being highly active against *Citrobacter* spp., *Enterobacter* spp., *E. coli*, *K. pneumoniae*, *Proteus* spp., and *Yersinia* spp. Unlike aminopenicillins, mecillinam has little activity against Gram-positive bacteria and none against *P. aeruginosa* (Table 7.5). It has high affinity only for PBP2, the enzyme-mediating cylindrical growth in Gram-negative rods. Mecillinam is synergistic with many beta-lactamase inhibiting drugs. It is inactivated by many beta-lactamases, but many ampicillin-resistant Enterobacteriaceae are susceptible; its efficacy against some beta-lactamase producing bacteria is because of its rapid penetration of cells as well as its low affinity for some of their degradative enzymes. Oral absorption is poor; and in part for this reason, this drug has not been used in veterinary medicine. Mecillinam may have potential for use in veterinary medicine for infections caused by susceptible Enterobacteriaceae, at a human dosage in the range of 5–10 mg/kg TID IM.

Group 5: Antipseudomonal Penicillins. Carboxypenicillins: Carbenicillin and Ticarcillin

Carbenicillin was the first penicillin with good activity against *P. aeruginosa* and *Proteus* (Table 7.2), but has

now been largely replaced by the more active ticarcillin, azlocillin, and piperacillin. It is administered IV. Two esters (carindacillin, carfecillin) are available for oral administration for urinary tract infections caused by *Proteus* or *P. aeruginosa*. Ticarcillin has a spectrum of activity similar to carbenicillin. It is active against most *E. coli* and *Proteus* and more active than carbenicillin against *P. aeruginosa* (Table 7.5). Most *Klebsiella*, *Citrobacter*, and *Serratia* are resistant; all *Enterobacter* are resistant. Ticarcillin is generally reserved for *P. aeruginosa* infections but is less active than azlocillin or piperacillin. It is administered IV.

Because of the expense of carbenicillin and ticarcillin, the high dosages required, IV administration, and general lack of clinical application, it is unlikely that carbenicillin and ticarcillin will be used for parenteral treatment of *Pseudomonas* or other infections in animals. These drugs have potential use in the local treatment of *P. aeruginosa* infections caused by otherwise resistant bacteria, such as otitis externa in dogs, bovine mastitis, ulcerative keratitis, metritis in mares, and otherwise resistant urinary tract infections. Ticarcillin is licensed in the United States for the treatment of uterine infections in mares caused by beta-hemolytic streptococci (6 gm in 250–500 ml by intrauterine infusion at estrus once daily for 3 days). For this purpose, ticarcillin has no advantage over benzyl penicillin and should be reserved for infections caused by *P. aeruginosa* and other susceptible Gram-negative bacteria. Ticarcillin was administered IV to a foal (110 mg/kg, QID) in the effective treatment of bacterial arthritis (Sweeney and Markel, 1984). A parenteral (IM) dosage suggested for dogs is 25–40 mg/kg every 6–8 hours; IV-administered drug should be given every 4–6 hours. Ticarcillin (15–25 mg/kg, IV, every 8 hours) has been used successfully, combined with topical administration, in the treatment of otitis externa in dogs caused by otherwise-resistant *P. aeruginosa* (Nuttall, 1998). Because of the danger of *P. aeruginosa* developing resistance, these agents are probably best used in conjunction with a broad-spectrum aminoglycoside or beta-lactamase inhibitor.

Group 5: Antipseudomonal Penicillins. Ureidopenicillins: Azlocillin, Mezlocillin, and Piperacillin

The expanded spectrum of activity of the antipseudomonal penicillins results from their interaction with

PBPs other than those that bind aminopenicillins, their increased penetration of Gram-negative bacteria, and their resistance to some species-specific chromosomal beta-lactamases. Ureidopenicillins bind PBP3, septal murein synthetase. They have increased activity against Gram-negative bacteria compared to carboxy- or aminobenzylpenicillins, notably against *Klebsiella* and *P. aeruginosa* (see Tables 7.2 and 7.5), and increased activity against *B. fragilis*.

Mezlocillin is more active than azlocillin against Enterobacteriaceae, although resistance is not infrequent because the bacteria are susceptible to common beta-lactamases (Table 7.5). Most *Enterobacter* and *Serratia* are resistant. Piperacillin combines the spectrum and is more active than both. It inhibits over 95% of *P. aeruginosa* and many Enterobacteriaceae and is active against many anaerobes, including many *B. fragilis*. Piperacillin is the most active broad-spectrum penicillin but is also susceptible to some common beta-lactamases, as well as to the penicillinase of *S. aureus*. Ureidopenicillins may be combined with beta-lactamase inhibitors (e.g., piperacillin with tazobactam, Chapter 9) or with aminoglycosides. There is incomplete cross-resistance among ureidopenicillins and carboxypenicillins.

Ureidopenicillins are administered IV, although azlocillin may be administered by (painful) IM injection. Expense limits their application. Clinical applications are probably limited to treatment of *P. aeruginosa* infections and, combined with an amino-

glycoside or beta-lactamase inhibitor, to serious infections caused by Gram-negative bacteria in immunocompromised hosts.

Bibliography

- Nuttall TJ. 1998. Use of ticarcillin in the management of canine otitis externa complicated by *Pseudomonas aeruginosa*. J Small Anim Pract 39:165.
Sweeney CR, Markel MD. 1984. Ticarcillin therapy in a foal with septic arthritis. Mod Vet Pract 6 5:841.

Group 6: Beta-lactamase Resistant Penicillins: Temocillin

Temocillin is ticarcillin modified by the addition of a 6 α -methoxy group to increase resistance to beta-lactamase. Temocillin's high activity against Enterobacteriaceae is at the expense of resistance by *Pseudomonas*, *B. fragilis*, and Gram-positive bacteria. More than 90% of Enterobacteriaceae are inhibited at ≤ 8 μ g/ml. It is stable to expanded-spectrum, plasmid-mediated beta-lactamases that inactivate third-generation cephalosporins. Temocillin has a long half-life (4.5 hours) in humans, allowing for once-daily dosage. Temocillin has many potential applications but its use, like that of the antipseudomonal penicillins, is limited by expense and the need for IV administration. There are no reports in the veterinary literature of its use in animals.

Beta-lactam Antibiotics: Cephalosporins

John F. Prescott

General Considerations

In cephalosporins, the beta-lactam ring is attached to a 6-membered dihydrothiazine ring, with the effect that the cephalosporin nucleus is inherently more resistant to beta-lactamases than is the penicillin nucleus (Figure 8.1). The 7-aminocephalosporanic acid molecule also provides more sites than the aminopenicillanic acid molecule for manipulation in the production of semisynthetic drugs. Changes at position 7 (R1) alter beta-lactamase stability and antibacterial properties particularly while changes at position 3 (R2) tend to alter metabolic stability and pharmacokinetic properties. True cephalosporins contain the common 7-aminocephalosporanic acid of *Cephalosporium acremonium*, whereas cephamycins are derived from *Streptomyces* species (cefotetan, cefoxitin) or are synthetic derivatives produced by substituting oxygen for sulfur (latamoxef).

Cephalosporins in general have the advantages of beta-lactamase stability, good activity against target proteins (PBPs), and good ability to penetrate bacterial cell walls. Although they may be active against a wide range of organisms, such activities are not uniform and there are subtle differences between the different molecules. Pharmacokinetically, cephalosporins are generally similar and have typical beta-lactam properties: They sometimes require parenteral injection, have short (1–2 hours) half-lives, and are usually excreted through the kidneys in the urine. They are bactericidal, relatively nontoxic, and can be used in many penicillin-sensitive individuals.

Classification

Cephalosporins have a wide range of antibacterial activity but show considerable diversity in their antibac-

terial properties. One approach to classification has been chronological or historical, with the different cephalosporins introduced since 1975 being described somewhat arbitrarily as “generations” (Tables 8.1, 8.2). Such language implies that each generation gained another general level of advantage over the previous one. More typically, some advantage(s) are added at the expense of one or more others. Differences among the generations are subtle but important.

Cephalosporins were originally introduced (first generation) for the treatment of penicillinase-resistant staphylococcal infections, with the advantage that these drugs also had a similar spectrum of activity against Gram-negatives to that of the extended spectrum aminobenzylpenicillins. Alterations of the side-chains on the 7-aminocephalosporanic acid nucleus and the discovery of the cephamycins led to increasing stability against the beta-lactamases of Gram-negative bacteria, including those of *Bacteroides fragilis* and *Pseudomonas aeruginosa*. This increase in stability is usually at the expense of decreasing activity against Gram-positive bacteria and results in pharmacokinetic differences. Because of the inadequacies of classification as generations, an expanded classification has been developed on the basis of antimicrobial activity, including beta-lactamase stability and pharmacological properties (Wise, 1997) (Table 8.1). This classification will be followed here.

The generations are broadly characterized as follows.

- First generation: primarily Gram-positive antibacterial activity, administered parenterally (IV, IM, SC) or in some cases orally.
- Second generation: Gram-positive and Gram-negative antibacterial activity, administered by all routes.

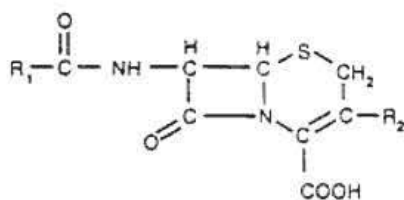


Figure 8.1. Structural formula of the cephalosporin nucleus.

- Third generation: decreased Gram-positive but increased Gram-negative antibacterial activity, administered parenterally and in a very few cases orally.
- Fourth generation: increased Gram-positive and Gram-negative antibacterial activity, administered by all routes.

Antimicrobial Activity

The mechanism of action of the cephalosporins is that of beta-lactam antibiotics (Chapter 7). For susceptibility testing, cephalothin is the class drug for group 1 and 2, first generation, cephalosporins. For groups 3-7, second to fourth generation cephalosporins, there is no class representative. For susceptibility testing of Enterobacteriaceae, cefotaxime can usually substitute for ceftazidime, ceftizoxime, and ceftriaxone (and vice versa) and cefamandole for cefonicid and cefuroxime (and vice versa). For *P. aeruginosa*, cefoperazone will substitute for ceftazidime (and vice versa) and cefotaxime for ceftriaxone and latamoxef (and vice versa).

Cephalosporins are usually active against beta-hemolytic streptococci and against beta-lactamase producing staphylococci, but not against methicillin (oxacillin) resistant staphylococci. Most enterococci are resistant. Among Enterobacteriaceae, in the absence of acquired resistance, *E. coli* and *Salmonella* are susceptible, as are some *Proteus* and *Klebsiella* spp. Fourth generation, group 7, cephalosporins are effective against Enterobacteriaceae and other Gram-negative bacteria resistant to earlier generations of cephalosporins because of acquired beta-lactamase based resistance. Susceptibility among common Gram-negative aerobic species such as *Haemophilus* and *Pasteurella*, including beta-lactamase producers, is usual. Only third generation antipseudomonal (group 6) and fourth generation (group 7) cephalosporins are effective against *P. aeruginosa*. Mycobacteria are resistant. Against nonsporeforming anaerobic bacteria, activity is variable and resembles that of aminobenzylpenicillins. Cefoxitin is notably resistant to beta-lactamase producing anaerobes, including *B. fragilis*.

Resistance to Cephalosporins

The three basic mechanisms of resistance to cephalosporins are PBP modification, reduced permeability and increased efflux, and enzymatic inactivation by beta-lactamases. Of these, the most important is beta-lactamase production, with more than 400 distinct beta-lactamases now recognized. Their importance is both because of the large number of different beta-

Table 8.1. Classification of cephalosporins into groups (and generations) based on route of administration and antibacterial activity.

Group	Characteristics	Examples
1 (First generation)	Parenteral; resistant to staphylococcal beta-lactamase; sensitive to enterobacterial beta-lactamase; moderately active.	Cephacetrile, cephaloridine, cephalothin, cephapirin, cephaloxin
2 (First generation)	Oral; resistant to staphylococcal beta-lactamase; moderately resistant to some enterobacterial beta-lactamase; moderately active.	Cefadroxil, cephradine, cephalixin
3 (Second generation)	Parenteral and oral; resistant to many beta-lactamases; moderately active.	Cefaclor, cefotetan, cefoxitin, cefuroxime, cefamandole
4 (Third generation)	Parenteral; resistant to many beta-lactamases; highly active.	Cefotaxime, Ceftizoxime, Ceftriaxone, Ceftiofur, Latamoxef
5 (Third generation)	Oral; resistant to many beta-lactamases; highly active.	Cefetamet, cefixime, cefpodoxime
6 (Third generation)	Parenteral; resistant to many beta-lactamases; active against <i>Pseudomonas aeruginosa</i> .	Cefoperazone, cefsulodin, ceftazidime
7 (Fourth generation; included with group 6 in some schemes)	Parenteral; resistant to staphylococcal, enterobacterial and pseudomonal beta-lactamases; highly active.	Cefepime, cefquinome, cefpirome

Classification is that of Wise (1997). By convention, cephalosporins discovered before 1975 are spelled with a "ph" and after 1975 with an "f".

Table 8.2. Relative activity of cephalosporins against selected opportunist bacteria^a

Drug	Generation	<i>S. aureus</i> ^b	<i>E. coli</i> , <i>Klebsiella</i> , <i>Proteus</i>	<i>Enterobacter</i>	<i>Pseudomonas</i> <i>aeruginosa</i>	<i>Bacteroides</i>	Other anaerobes
Cephalothin	1	+++	++	—	—	—	+
Cefuroxime	2	++	+++	—	—	—	+
Cefoxitin	2	+	+++	+	—	++	++
Cefotaxime	3	++	+++	+	—	+	++
Ceftazidime	3	+	+++	++	+++	—	—
Ceftriaxone	3	+	+++	+	—	—	+
Cefepime	4	++	+++	+++	+++	—	+

^a +++ highly active; ++ moderately active; + limited activity; — no clinical activity. Susceptibilities for individual isolates may vary.

^b Methicillin-susceptible *Staphylococcus aureus*. Table adapted from Marshall and Blair (1999).

lactamases that have been selected by the widespread use of extended spectrum cephalosporins and because genes for these beta-lactamases are often transmissible. The topic has been the subject of a number of excellent reviews (Livermore, 1998; Heritage et al., 1999; Bradford, 2001; Bush, 2001; Rupp and Fey, 2003).

PBP Modifications

Modification of normal PBPs occurs after transformation of bacteria by fragments of PBP DNA and their homologous recombination with existing PBP genes to produce new “mosaic” PBPs with low affinity for beta-lactams. This has been extensively described for some important human pathogens but is not recognized in bacterial pathogens of animals. Other important forms of PBP modification include acquisition of extra “by-pass” (insensitive) PBP genes by methicillin-resistant *Staphylococcus aureus* or by *Enterococcus faecium*, although this has not yet been described in animal pathogens (Livermore, 1998).

Reduced Uptake and Increased Efflux

Resistance to cephalosporins occurs from reduced production of the porins that beta-lactams use to penetrate Gram-negative bacteria. In some cases it is also the result of a periplasmic beta-lactamase enzyme. Such reduced uptake may be mediated by an efflux mechanism which gives broad-spectrum, cross-drug class resistance.

Beta-lactamases

There has been an astonishing, continuing evolution of these enzymes in response to antimicrobial selection pressure and subsequent widespread plasmid- or

transposon-mediated dissemination throughout Gram-negative bacterial populations. Most (class A, C, D molecules) are serine esterases but some (class B) are zinc metalloproteases. Beta-lactamases and their classification are discussed in more detail in Chapter 9 (Table 9.1).

The ability of transposable elements to move beta-lactamases from chromosomes to plasmids (and back again, and between different plasmids), as well as recombination processes involving integrons, means that the earlier designations of beta-lactamases as either chromosomal- or plasmid-mediated is anachronistic. However, the extent or degree of resistance provided by a beta-lactamase is a function both of its activity and its quantity, which in turn may depend on plasmid copy numbers or the extent to which chromosomal enzymes can be induced.

First-generation Cephalosporin Beta-lactamases

The development of aminopenicillins (e.g., ampicillin) in the early 1960s broadened the activity of penicillins against Gram-negative bacteria, particularly *Escherichia coli*. It was followed by the development and spread of plasmid-mediated beta-lactamases, notably TEM-1 (now a common feature of *E. coli*), as well as SHV-1 and OXA-1 (Livermore, 1998). The first-generation cephalosporins developed at this time were not only resistant to staphylococcal beta-lactamases, which ampicillin was not, but also had a spectrum of activity against Gram-negative aerobes slightly broader than that of the aminopenicillins. However, they were susceptible to the same plasmid-mediated beta-lactamases as ampicillin and also lacked its activity against inducible functional group 1 AmpC enzymes.

Second-generation Cephalosporin Beta-lactamases

In the search for beta-lactams resistant to the beta-lactamases emerging and conferring resistance in the late 1960s, cephalosporins with enhanced beta-lactamase stability were more readily developed than amino- or carboxy-penicillins. These second-generation cephalosporins were more stable to TEM-1 and against some AmpC-inducible enteric bacteria such as *E. coli*. As noted, the first cephamycin, cefoxitin, was also found to be uniquely stable to the chromosomal beta-lactamases of *Bacteroides* spp., including *B. fragilis*. However, these new drugs remained ineffective against important Gram-negative aerobic pathogens such as *P. aeruginosa*.

Third-generation Cephalosporin Beta-lactamases

The third-generation drugs developed in the 1970s and 1980s had considerably enhanced activity against Enterobacteriaceae, including TEM-1, TEM-2, and SHV-1 plasmid-containing strains as well as some *P. aeruginosa*. Unlike earlier drugs, they had stability against chromosomal beta-lactamases of *Klebsiella* spp. and against functional group 1 AmpC-inducible enteric bacteria because of their weak induction of these enzymes. These enhanced activities were at the expense of reduced activity against staphylococci.

Unfortunately, resistance has emerged in the Gram-negative bacterial targets of these drugs and is becoming increasingly widespread through plasmid and transposon transmission, particularly among the Enterobacteriaceae (*Enterobacter* spp., *E. coli*, *Morganella morganii*, *Proteus* spp., and *Salmonella*). Resistance has also spread to *Burkholderia* spp. and to *P. aeruginosa*. The major types of beta-lactamase that are increasing in global prevalence among opportunist pathogens are the plasmid-encoded functional group 1 cephalosporinases, the group 2b extended-spectrum beta-lactamases (ESBLs), and the group 3 metallo-beta-lactamases (Table 9.1). Of these, the greatest increase is occurring in the ESBLs.

AmpC Hyperproducers

The hyperproduction of AmpC beta-lactamases occurs most often in bacterial opportunist pathogens that are relatively unusual in animals, notably *Enterobacter* spp. and *Citrobacter freundii*. Paradoxically, although third-generation cephalosporins are weak inducers of these enzymes, they are actually effective in killing organisms producing these enzymes. However,

they are ineffective when the enzymes are produced in large amounts by hyperproducers, which are those that have a mutation in the gene encoding the peptidoglycan recycling enzyme AmpD. Such "derepressed mutants" resistant to all cephalosporins (and to clavulanic acid and other beta-lactamase inhibitors) may emerge during therapy of infections caused by these two genera (in sites other than the urinary tract) and may be particularly problematic in hospital settings. More seriously, AmpC hyperproduction can become encoded by high copy number plasmids (FOX, MIR, MOX, CMY-beta-lactamase families or types) and mobilized to other Gram-negative bacteria, notably *E. coli* and *Klebsiella* spp., in which the new set of group 1 cephalosporinases may be additive with endogenous non-group 1 beta-lactamases (Bush, 2001).

In recent years, there has been increasing spread of a family of CMY2-encoding plasmids in animals. Hospital acquired infection from multiresistant *E. coli* producing the cephamycinase-encoding gene CMY2 was described in 23 dogs with nosocomial infections in a veterinary hospital in the United States (Sanchez et al., 2002), with the same isolate detected from the intensive care unit and surgical wards. Many of these isolates were also resistant to florfenicol, and the *flo* and *bla*_{CMY2} genes were transferable, probably by a transposon. Additional resistance to spectinomycin and sulfonamides in the isolates was also provided by integrons (Sanchez et al., 2002). CMY2 AmpC beta-lactamase plasmids are common in, and move between, *E. coli* and *Salmonella* isolated from food animals and people (Winokur et al., 2001; Zhao et al., 2001), and have recently spread into *Salmonella* Newport (Zhao et al., 2003). Their further spread into and dissemination by animals is anticipated.

Extended-spectrum Beta-lactamases

ESBLs contain the greatest number of distinct beta-lactamase enzymes that are variants of the broad-spectrum TEM and SHV beta-lactamases, all of which are plasmid or transposon mediated. Currently there are over 130 TEM-type and over 40 SHV-type ESBLs (Table 9.1). These enzymes produce resistance by hydrolyzing the oxyimino-aminothiazole-containing beta-lactams (aztreonam, cefotaxime, ceftazidime, and to some extent cefepime, as well as earlier generation cephalosporins). By contrast, the α -methoxycephalosporins (cefoxitin, cefotetan, latamoxef) and imipenem are stable to these enzymes. There are dif-

ferences between different ESBLs in the rate at which they hydrolyze different cephalosporins. For example, TEM-12 and SHV-2 ESBLs hydrolyze cephalosporins slowly, so that third-generation cephalosporin treatment may still be effective; however, a second single nucleotide mutation in the TEM-12 beta-lactamase gene produces high level resistance (Bradford et al., 1994). Other plasmid-mediated ESBLs not closely related to the TEM and SHV families include the CTX-M family, that preferentially hydrolyze cefotaxime (and cefepime). These number at least 40 distinct enzymes, the cefotaximases of the SFO-1 and BES-1 types, and the PER, VEB, TLA-1 and GES/IBC types that preferentially hydrolyze ceftazidime (Bonnet 2004). Third-generation cephalosporin beta-lactamase-producing Enterobacteriaceae are causing infections in animals (Teshager et al., 2000; Warren et al., 2001; Bríñas et al., 2002; Féria et al., 2002; Sanchez et al., 2002). The CTX-M-type ESBLs in particular are expanding among *Salmonella*, in some cases being associated with *sulI*-type integrons on complex plasmids (Miriagou et al., 2004).

In human medicine, ESBL-producing bacteria most often cause infection in severely ill hospitalized patients in the intensive care unit, but outbreaks have also been described in nursing homes, pediatric units, and other hospital settings. Such outbreaks require institution of rigorous infection control procedures and monitoring. In addition, use of extended-spectrum beta-lactams must be restricted by switching to other drug classes for empirical therapy of serious infections (Rupp and Frey, 2003).

Most of the third-generation cephalosporin beta-lactamase-producing bacterial infections described in companion animals were acquired in veterinary teaching hospitals. For example, nine of ten ESBL-producing *E. coli* involved in canine infections were identified in a hospital setting in Australia (Warren et al., 2001), although the molecular basis of the ESBL was not investigated.

Group 3 Metallo-beta-lactamases

Metallo-beta-lactamases have emerged in the last decade as important beta-lactamases of nonfermenting Gram-negative bacteria (*Aeromonas* spp., *P. aeruginosa*). The genes for these enzymes (IMP, SPM, VIM types) can be transferred through plasmids to Enterobacteriaceae such as *Enterobacter* and *Klebsiella*. Enzymes of the IMP and VIM types can degrade vir-

tually all beta-lactams other than monobactam (Luzzaro et al., 2004). Some of these beta-lactamases are carried on integrons that encode multiple drug resistance genes (Weldhagen, 2004).

Pharmacokinetic Properties

The pharmacokinetic and drug disposition characteristics of cephalosporins are typical of beta-lactams (Chapter 7), with an elimination half-life of 1–2 hours. Some drugs, however, such as cefotetan and ceftriaxone, have significantly longer half-lives. Group 2 (second generation) and 5 (third generation) oral cephalosporins are well absorbed after oral administration, which may be enhanced by formulation as prodrugs which are metabolized to the active compound in the body. Some fourth generation cephalosporins can be administered orally to monogastrates. Clearance is through the kidney except for some drugs with high molecular weight and protein binding, such as cefoperazone, which are largely excreted in the bile.

Drug Interactions

Cephalosporins are synergistic with aminoglycosides, and combined in the treatment of infections in neutropenic human patients.

Toxicity and Adverse Effects

Cephalosporins are among the safest antimicrobial drugs. They have the safety associated with penicillins, although individual drugs may have specific adverse effects. For example, bleeding disorders associated with hypoprothrombinemia and platelet abnormalities may be exacerbated by some newer cephalosporins. The broad-spectrum of antibacterial activity of second to fourth generation drugs may cause overgrowth ("superinfection") by inherently resistant bacteria, including *Clostridium difficile*, which no longer have to compete with the normal microbial flora. The emergence of multiresistant enterococci as nosocomial infections in human intensive care units is an example of this effect. Gastrointestinal disturbances are common adverse effects, particularly with drugs excreted in bile. Human patients allergic to penicillins are sometimes (5–8%) also allergic to cephalosporins. Many second and third generation drugs are painful on injection and are therefore usually administered IV. Orally administered third generation (group 5) cephalosporins are now available.

Dosage Considerations

As with all beta-lactams, the treatment efficacy of the cephalosporins is time-dependent; it is correlated with maintaining serum and tissue concentrations of drug \geq MIC for most or all of the dosing interval.

Clinical Usage

Cephalosporins are an important class of antimicrobial agent with widespread potential use. First generation cephalosporins have a spectrum of activity and clinical use similar to that of extended spectrum aminopenicillins, with the addition of resistance to staphylococcal beta-lactamase. First generation oral cephalosporins are used in the treatment of canine *S. intermedius* pyoderma and urinary tract infections, as well as bovine *S. aureus* and streptococcal mastitis.

Second and some third generation (groups 3, 4) parenteral cephalosporins are used to treat infections caused by bacteria resistant to first generation drugs. For example, ceftiofur, which has antimicrobial characteristics between second and third generation cephalosporins, is used in animals to treat systemic infections caused by Gram-negative aerobes, including *E. coli*, *Pasteurella* and *Salmonella*. Cefoxitin has a special place in the treatment of mixed aerobic-anaerobic infections.

The antipseudomonal cephalosporins (group 6) are used exclusively in the treatment of *P. aeruginosa* infections. Other third (group 5) and the fourth generation cephalosporins are usually (but not always) reserved in human medicine for the treatment of hospital-based bacterial infections resistant to earlier cephalosporins or alternative antimicrobial drugs.

Broad-spectrum bactericidal activity (at concentrations \leq 4 MIC) may be a drawback of newer cephalosporins, since it is associated with resistant bacterial superinfection and gastrointestinal disturbance. Widespread use of third generation cephalosporins in human medicine may a primary cause of the resistance crisis, and has been associated with the striking emergence and dissemination of multiple forms of beta-lactamases observed in recent years. Second and third generation cephalosporins are not usually first choice antimicrobial agents in animals but rather should be reserved for use where susceptibility testing indicates that alternatives are not available and for serious, life-threatening infections caused by Gram-negative bacteria.

Bibliography

- Barton MD. 2001. Is it reasonable to use third generation cephalosporins to treat animals? *Aust Vet J* 79:620.
- Bonner R. 2004. Growing group of extended-spectrum β -lactamases: the CTX-M enzymes. *Antimicrob Agents Chemother* 48:1.
- Bradford PA. 2001. Extended-spectrum β -lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev* 14:933.
- Briñas L, et al. 2002. β -lactamases in ampicillin-resistant *Escherichia coli* isolates isolated from foods, humans, and healthy animals. *Antimicrob Agents Chemother* 46:3156.
- Bush K. 2001. New β -lactamases in Gram-negative bacteria: Diversity and impact on the selection of antimicrobial therapy. *Clin Infect Dis* 32:1085.
- Féria C, et al. 2002. Patterns and mechanisms of resistance of β -lactams and β -lactamase inhibitors in uropathogenic *Escherichia coli* isolated from dogs in Portugal. *J Antimicrob Chemother* 49:77.
- Heritage J, et al. 1999. Evolution and spread of SHV extended-spectrum β -lactamases in Gram-negative bacteria. *J Antimicrob Chemother* 44:309.
- Livermore DM. 1998. β -lactamase-mediated resistance and opportunities for its control. *J Antimicrob Chemother* 41: Suppl. D, 25.
- Marshall WF, Blair JE. 1999. The cephalosporins. *Mayo Clin Proc* 74:187.
- Miriabou V, et al. 2004. Expanded-spectrum cephalosporin resistance in non-typhoid *Salmonella*. *Int J Antimicrob Ag* 23:547.
- Pfizer Animal Health. 2004. Cephalosporins in Veterinary Medicine. 67 pp. New York: Pfizer, Inc.
- Rupp ME, Fey PD. 2003. Extended-spectrum β -lactamases (ESBL)-producing Enterobacteriaceae. *Drugs* 63:353.
- Sanchez S, et al. 2002. Characterization of multiresistant *Escherichia coli* isolates associated with nosocomial infections in dogs. *J Clin Microbiol* 40:3586.
- Teshager T, et al. 2000. Isolation of an SHV-12 beta-lactamase-producing *Escherichia coli* strain from a dog with recurrent urinary tract infections. *Antimicrob Agents Chemother* 44:3483.
- Warren AL, et al. 2001. Multi-drug resistant *Escherichia coli* with extended-spectrum β -lactamase activity and fluoroquinolone resistance isolated from clinical infections in dogs. *Aust Vet J* 79:621.
- Weldhagen GF. 2004. Integrons and β -lactamases. *Int J Antimicrob Ag* 23:556.
- Winokur PL, et al. 2001. Evidence of transfer of CMY-2 AmpC α -lactamase plasmids between *Escherichia coli* and *Salmonella* isolates from food animals and humans. *Antimicrob Agents Chemother* 45:2716.
- Wise R. 1997. β -lactams: cephalosporins. In: O'Grady F, et al (eds). *Antibiotics and Chemotherapy*. New York: Churchill Livingstone, p 202.
- Zhao S, et al. 2001. Identification and expression of cephamycinase blaCMY genes in *Escherichia coli* and *Sal-*

monella isolates from food animals and ground meat. Antimicrob Agents Chemother 45:3647.

Zhao S, et al. 2003. Characterization of *Salmonella enterica* serotype Newport isolated from humans and food animals. J Clin Microbiol 41:5366.

Group 1 First-generation Cephalosporins: Cefacetrile, Cephapirin, Cephaloridine, Cefazolin, Cephalothin

First-generation, group 1, parenteral cephalosporins share with oral first-generation cephalosporins high activity against Gram-positive bacteria including beta-lactamase-producing *S. aureus*; moderate activity against certain nontransferable, beta-lactamase-producing, Gram-negative Enterobacteriaceae and fastidious Gram-negatives; and no activity against *Enterobacter* spp., *P. aeruginosa*, and *Serratia* spp., among others. For susceptibility testing, cephalothin is the class drug but cefazolin may also be tested since it is more active against Gram-negative bacteria. Activity is shown for selected bacteria in Tables 8.2 and 8.3.

Acquired resistance is common in Gram-negative but rare in Gram-positive bacteria. Methicillin-resistant *S. aureus*, discussed in Chapter 7, are resistant to all beta-lactam drugs including cephalosporins.

Pharmacokinetic Properties

IM or SC injection results in rapid absorption with high bioavailability. There is widespread distribution in extracellular fluids but poor penetration across biological membranes (including into the udder) and physiological barriers (such as the blood-brain barrier). Cephalothin and cephapirin are metabolized into the less active desacetyl derivatives. The majority of drug is rapidly eliminated in the urine, and tubular secretion (but not glomerular filtration) can be inhibited by probenecid to slow clearance from the body. The specific mechanism of renal excretion varies with the agent. Half-life is less than 1 hour.

Toxicities and Side Effects

Intramuscular injections of cephalothin are painful, so it is not administered by this route. Non-dose-related hypersensitivity, fever, skin rash, and eosinophilia occur infrequently. At very high doses, nephrotoxicity caused by acute tubular necrosis may occur. Because of this, cephaloridine is no longer available for clinical use.

Table 8.3. Activity ($\mu\text{g/ml}$) of first-generation cephalosporins (cephalothin) against selected bacteria isolated from animals.

Organism	MIC ₅₀	MIC ₉₀
Gram-positive aerobes		
<i>Arcanobacterium pyogenes</i>	0.5	4
<i>Bacillus anthracis</i>	0.25	0.5
<i>Corynebacterium pseudotuberculosis</i>	≤ 1	≤ 1
<i>Erysipelothrix rhusiopathiae</i>	0.25	0.5
<i>Enterococcus</i> spp.	>32	>32
<i>Listeria monocytogenes</i>	2	4
<i>Nocardia asteroides</i>	64	>128
<i>Rhodococcus equi</i>	>128	>128
<i>Staphylococcus aureus</i>	0.5	1
<i>Streptococcus agalactiae</i>	≤ 0.12	0.5
<i>Streptococcus canis</i>	≤ 0.12	0.25
<i>Streptococcus uberis</i>	0.5	2
Gram-positive anaerobes		
<i>Actinomyces</i> spp.	0.06	0.12
<i>Clostridium perfringens</i>	0.5	1
<i>Clostridium</i> spp.	0.5	1
Gram-negative aerobes		
<i>Actinobacillus</i> spp.	≤ 1	16
<i>Bordetella avium</i>	≤ 1	≤ 1
<i>Bordetella bronchiseptica</i>	16	64
<i>Brucella canis</i>	8	16
<i>Camylobacter jejuni</i>	≤ 512	≤ 512
<i>Escherichia coli</i>	8	64
<i>Klebsiella pneumoniae</i>	4	>64
<i>Leptospira</i> spp.	1	8
<i>Mannheimia haemolytica</i>	1	8
<i>Pasteurella multocida</i>	1	8
<i>Pseudomonas aeruginosa</i>	>64	>64
<i>Salmonella</i> spp.	2	8
Gram-negative anaerobes		
<i>Bacteroides fragilis</i>	>32	>32
<i>Bacteroides</i> spp.	16	>32
<i>Fusobacterium</i> spp.	0.5	≤ 1
<i>Porphyromonas</i> spp.	1	16

Bacteria with MIC $\leq 8 \mu\text{g/ml}$ are susceptible, $16 \mu\text{g/ml}$ moderately susceptible, and $\geq 32 \mu\text{g/ml}$ are resistant.

Administration and Dosage

Recommended dosages are shown in Table 8.4. Because of the margin of safety, a range of dosages can be used depending on the MIC of susceptible bacteria.

Clinical Applications

Clinical applications of parenteral first-generation cephalosporins have become fewer with the development of beta-lactamase-stable cephalosporins. Applications are as described for oral cephalosporins below,

Table 8.4. Parenteral dosages (IV, IM, SC) of Group 1 parenteral cephalosporins.

Species	Drug	Dose (mg/kg)	Interval (h)	Comments
Dog, cat	Cefazolin	15-30	4-8	IM, IV
	Cephalothin	10-30	4-8	IV only (painful IM)
Horse	Cefazolin	15-20	8	
	Cephalexin	10	8-12	
	Cephalothin	10-25	4	
	Cephapirin	20-30	4-8	Highly susceptible, e.g. <i>S. aureus</i>
Cattle, sheep	Cefazolin	15-20	12	Poor udder penetration
	Cephapirin	10	8-12	Poor udder penetration

which are used extensively in small animal medicine. These drugs have been used extensively in prophylaxis of surgical wound infections in human patients and are used for this purpose in dogs and cats. Cefazolin has been suggested for administration (20 mg/kg IV) at the time of surgery, repeated SC 6 hours later (Rosin et al. 1993).

In dogs and cats, parenteral first generation drugs might be used to establish high tissue levels rapidly before using an oral cephalosporin. In horses, an important indication would be parenteral treatment of non-MRSA *S. aureus* infections. In the absence of susceptibility testing, their use in treating infections caused by Gram-negative Enterobacteriaceae is not generally recommended since activity is unpredictable (as is the case also for aminobenzylpenicillins). In cattle, these cephalosporins are widely used in the treatment and prevention (dry-cow therapy) of mastitis caused by the Gram-positive cocci, as alternatives to pirlimycin, cloxacillin, and penicillin-novobiocin combination. Administration is by the intramammary route.

Bibliography

- Gagnon H, et al. 1994. Single-dose pharmacokinetics of cefazolin in bovine synovial fluid after intravenous regional injection. *J Vet Pharm Ther* 17:31.
- Marcellin-Little DJ, et al. 1996. Pharmacokinetic model for cefazolin distribution during total hip arthroplasty in dogs. *Am J Vet Res* 57:720.
- Petersen SW, Rosin E. 1995. Cephalothin and cefazolin in vitro antibacterial activity and pharmacokinetics in dogs. *Vet Surg* 24:347.
- Rosin E, et al. 1993. Cefazolin antibacterial activity and concentrations in serum and the surgical wound in dogs. *Am J Vet Res* 54:1317.
- Silley P, et al. 1988. Pharmacokinetics of cephalexin in dogs and cats after oral, subcutaneous and intramuscular administration. *Vet Rec* 122:15.
- Soback S, et al. 1987. Clinical pharmacology of cefazolin in calves. *J Vet Med* 34A:25.

Group 2 Oral First-generation Cephalosporins: Cefadroxil, Cephadrine, Cephalexin, and Cephaloglycin

First-generation, group 2, oral cephalosporins share the characteristics of the group 1 parenteral cephalosporins in high activity against Gram-positive bacteria including beta-lactamase-producing *S. aureus*; moderate activity against certain nontransferable, beta-lactamase-producing, Gram-negative Enterobacteriaceae and fastidious Gram-negatives; and no activity against *Enterobacter* spp., *P. aeruginosa*, and *Serratia* spp., among others (Table 8.2).

Antimicrobial Activity

Antimicrobial activity of oral cephalosporins is similar to that of aminopenicillins, with the addition of resistance to the beta-lactamase of *S. aureus*.

Good susceptibility ($\leq 8 \mu\text{g/ml}$) is shown by many Gram-positive bacteria including *S. aureus*, streptococci (not enterococci), *Actinomyces* spp., *Bacillus* spp., *Corynebacterium* spp., *E. rhusiopathiae*, and most *L. monocytogenes* (Table 8.2). Susceptible anaerobes include some *Bacteroides*, most *Clostridium* spp., and most *Fusobacterium* spp. Susceptible Gram-negative aerobes include fastidious organisms such as *Bordetella avium*, *Haemophilus* spp., and *Pasteurella* spp.

Variable susceptibility due to acquired resistance is shown by *E. coli*, *Klebsiella* spp., *Proteus* spp., and *Salmonella* spp.

Moderate susceptibility (16 µg/ml). *Actinobacillus* spp., *Brucella* spp., some *Bacteroides* spp.

Resistance (≥ 32 µg/ml). *Acinetobacter* spp., *Bacteroides fragilis*, *Bordetella bronchiseptica*, *Campylobacter* spp., *Citrobacter* spp., *Enterobacter* spp., *Nocardia* spp., *Enterococcus faecalis*, *P. aeruginosa*, *R. equi*, *Serratia* spp., *Yersinia* spp.

Antibiotic Resistance

Acquired resistance occurs in Gram-negative bacteria and is particularly important in Enterobacteriaceae.

Pharmacokinetic Properties

Oral cephalosporins have pharmacokinetic properties similar to penicillin V and the aminobenzylpenicillins. Generally they are rapidly and largely absorbed after oral administration in monogastrates. With the exception of cephadrine, these drugs are unaffected by the presence of food. Relatively wide distribution occurs in extracellular fluids but penetration across biological membranes is poor. Inflammation enhances passage across barriers. Half-lives are short, usually less than 1 hour, although cefadroxil has a longer half-life in dogs. Cephalosporins are largely excreted unchanged in urine. Plasma protein binding is low. Absorption in horses and ruminants is poor and highly erratic.

Drug Interactions

Oral cephalosporins are potentially synergistic with aminoglycosides although indications for such combinations would be unusual.

Toxicities and Side Effects

Cephalosporins are among the safest of antimicrobial drugs. Allergic reactions, including acute, anaphylactic hypersensitivity, are rare. In humans, the majority of allergic reactions are not cross-reactive with penicillin. A small proportion of human patients develop eosinophilia, rash, and drug fever. Vomiting and diarrhea may occur in a small proportion of monogastrates given oral cephalosporins.

Administration and Dosage

Recommended dosages are shown in Table 8.5. Oral cephalosporins should not be used in herbivores.

Table 8.5. Recommended dosages of oral cephalosporins.

Species	Drug	Dose (mg/kg)	Interval (h)
Dog, cat	Cefaclor	4-20	8
	Cefadroxil	22	12 (dogs) 24 (cats)
	Cefixime	5	12-24 (dogs)
	Cefpodoxime proxetil	5-10	24 (dogs)
	Cephalexin	30	12
Calves (preruminant)	Cephadrine	10-25	6-8
	Cefadroxil	25	12
	Cephadrine	7	12
	Cefadroxil	20-40	8
Horse (foals)	Cefpodoxime proxetil	10	6-12
	Cephadrine	25	6-8

Clinical Applications

Oral cephalosporins have similar applications to penicillinase-resistant penicillins and aminobenzylpenicillins in monogastric animals, so that they are widely used in small animal medicine. The cephalosporins are thus potentially useful in a variety of non-specific infections caused by staphylococci, streptococci, Enterobacteriaceae, and some anaerobic bacteria.

Cephalexin is frequently used long-term (30 days) in the treatment of chronic *S. intermedius* pyoderma in dogs. Prophylactic use on two consecutive days a week prevented recurrence of German Shepherd Dog pyoderma (Bell 1995). Chronic therapy of deep pyoderma is associated with increased antimicrobial resistance in *S. intermedius* isolates, which may be transferred to humans (Guardabassi et al., 2004).

Cephalexin has been described as the drug of choice for *K. pneumoniae* urinary tract infections (Ling and Ruby 1983), although a fluoroquinolone is now a better choice. Other applications for cephalexin include the treatment of abscesses and wound infections caused by susceptible organisms in dogs and cats.

Bibliography

- Bell A. 1995. Prophylaxis of German Shepherd recurrent furunculosis (German Shepherd dog pyoderma) using cephalexin pulse therapy. Aust Vet Pract 25:30.
- Campbell BG, Rosin E. 1998. Effect of food on absorption of cefadroxil and cephalexin in dogs. J Vet Pharm Ther 21:418.
- Duffee NE, et al. 1997. The pharmacokinetics of cefadroxil over a range of oral doses and animal ages in the foal. J Vet Pharm Ther 20:427.

- Guardabassi L, et al. 2004. Transmission of multiple antimicrobial-resistant *Staphylococcus intermedius* between dogs affected by deep pyoderma and their owners. *Vet Microbiol* 98:23.
- Ling GV, Ruby AL. 1983. Cephalexin for oral treatment of canine urinary tract infection caused by *Klebsiella pneumoniae*. *J Am Vet Med Assoc* 182:1346.
- Silley P, et al. 1988. Pharmacokinetics of cephalexin in dogs and cats after oral, subcutaneous and intramuscular administration. *Vet Rec* 122:15.
- Soback S, et al. 1987. Clinical pharmacokinetics of five oral cephalosporins in calves. *Res Vet Sci* 43:166.
- Thornton JR, Martin PJ. 1997. Pharmacokinetics of cephalexin in cats after oral administration of the antibiotic in tablet and paste preparations. *Aust Vet J* 75:439.
- Whittem T, Slacek B. 1996. Contrast between the pharmacokinetics of two formulations of cephalexin after intramuscular administration in cattle. *NZ Vet J* 44:145.

Group 3 Second-generation Cephalosporins: Cefaclor, Cefoxitin, Cefmetazole, Cefotetan, Cefuroxime, Cefamandole

Second-generation, group 3, cephalosporins have a wide spectrum of antibacterial activity largely because of their stability to a broad range of beta-lactamases. They are moderately active against Gram-positive bacteria. Cephamycins (cefotetan, cefoxitin) are products of *Streptomyces* rather than of *Cephalosporium* species and differ from cephalosporins in the presence of a methoxy group in the 7 position of the cephalosporin nucleus. Cephamycins are very stable to beta-lactamases, including those of *Bacteroides fragilis*, but like other second-generation drugs, are not active against *P. aeruginosa*.

Antimicrobial Activity

Cefoxitin is resistant to most bacterial beta-lactamases, although it penetrates Gram-negative bacteria relatively poorly. Antimicrobial activity is slightly broader and greater than that of cefazolin and other first-generation cephalosporins for Gram-negative bacteria and includes *Enterobacter* spp. and *Serratia* spp. Activity against Gram-positive bacteria is slightly less. Cefoxitin is stable to the beta-lactamase of *B. fragilis* and has good activity against this and other *Bacteroides*, *Porphyromonas* and *Prevotella* spp. *Pseudomonas aeruginosa*, enterococci, and some Enterobacteriaceae are resistant (Table 8.2). Cefotetan has the greatest activity of the 7-methoxy cephalosporins against Gram-negative bacteria but *P. aeruginosa* is re-

sistant. A proportion of *Citrobacter*, *Enterobacter*, and *Serratia* spp. isolates are resistant. Activity against anaerobes is similar to cefoxitin, but a proportion of *B. fragilis* are resistant. Cefmetazole has a spectrum of activity similar to cefoxitin but it is more active against Enterobacteriaceae.

Resistance

Stable derepression of inducible beta-lactamases associated with hyperproduction of AmpC beta-lactamases in certain Gram-negative pathogens is an important mechanism of resistance. Cefoxitin is a powerful beta-lactamase inducer and can therefore antagonize the effects of other beta-lactams. As described earlier, there has been increasing spread of a family of cephamycinase (CMY2)-encoding plasmids in animals, noted not only in hospital-acquired infections in companion animals (Sanchez et al., 2002) but also in *Salmonella* infections (Zhao et al. 2001, 2003).

Pharmacokinetic Properties

Pharmacokinetic properties and toxicities are similar to those of first-generation parenteral cephalosporins. With the exception of cefaclor, they are not absorbed following oral administration. Cefuroxime axetil is an ester of cefuroxime which is hydrolysed in the intestinal mucosa and liver to yield active drug, producing good bioavailability after oral administration. Excretion, which can be delayed by probenecid, is largely renal. Half-lives in cattle and horses are about 1 hour. The 3-hour half-life of cefotetan in humans allows twice daily dosing.

Toxicities and Adverse Effects

Second generation cephalosporins cause pain on IM injection and may cause thrombophlebitis when administered IV. Cefoxitin may cause hypoprothrombinemia and bleeding tendencies in human patients. Cefamandole in humans produces alcohol intolerance by blocking liver acetaldehyde dehydrogenase and may cause a coagulopathy associated with hypoprothrombinemia, which is reversible by vitamin K. For this latter reason, cefamandole is rarely if ever used in human medicine. Use in animals has been too limited to describe toxicities, but their broad antibacterial activity may lead to gastrointestinal disturbances and superinfection by resistant microorganisms, including yeasts. This has been particularly marked with cefuroxime axetil administered orally to human patients.

Table 8.6. Dosage of Groups 3 and 4 parenteral cephalosporins.

Species	Drug	Dose (mg/kg)	Interval (h)
Dog, cat	Cefotaxime IM (SC)	20-40	8 (SC 12)
	Cefoperazone IV, IM	20-25	6-8
	Cefoxitin (IV, IM, SC)	15-30	6-8
	Ceftiofur IM	2.2 (dogs)	24
	Ceftizoxime IV, IM	25-40	8-12
	Ceftriaxone IV, IM	25	12-24
	Cefuroxime IV	10-15	8-12
Cattle, sheep, goats	Ceftiofur IM	1.1-2.2	24
Cattle	Ceftiofur crystalline free acid, posterior ear	6.6	5 days
Horses	Cefotaxime IV	20-40	6-8
	Cefoxitin IV, IM	20	8
	Ceftiofur IM	2.2-4.4	24
	Ceftriaxone IV, IM	25	12 (not adults)
	Ceftiofur IM	3-5	24
Swine	Ceftiofur crystalline free acid IM	5.0	5 days

Administration and Dosage

Administration is usually IV because of pain associated with IM injection. Dosage in animals, which in some cases is empirical, is shown in Table 8.6. Cefaclor and cefuroxime axetil can be administered orally in monogastrates.

Clinical Applications

Clinical applications in animals are limited by the expense of these drugs, but may be similar to those identified in human medicine. Cefoxitin is valued particularly for its broad activity against anaerobes, especially *B. fragilis*, as well as against Enterobacteriaceae. In veterinary patients, cefoxitin is used in treatment of severe mixed infections with anaerobes (aspiration pneumonia, severe bite infections, gangrene, peritonitis, pleuritis) and prophylaxis for colonic surgery or ruptured intestine. Cefuroxime is available and effective for short-lasting dry-cow therapy and for treatment of clinical mastitis in lactating cows. In human medicine, cefuroxime axetil is given orally to treat otitis media and upper respiratory infections caused by susceptible bacteria. The widespread use of cephalosporins for these purposes may have been responsible for the extensive emergence of penicillin resistance in *Streptococcus pneumoniae*, an important human pathogen.

Bibliography

- Caprile KA. 1988. The cephalosporin antimicrobial agents: A comprehensive review. *J Vet Pharm Ther* 11:1.
- Perry CM, Brogden RN. 1996. Cefuroxime axetil. A review of its antibacterial activity, pharmacokinetic properties and therapeutic efficacy. *Drugs* 52:125.
- Petersen SW, Rosin E. 1993. In vitro antibacterial activity of cefoxitin and pharmacokinetics in dogs. *Am J Vet Res* 54:1496.
- Sanchez S, et al. 2002. Characterization of multidrug-resistant *Escherichia coli* isolates associated with nosocomial infections in dogs. *J Clin Microbiol* 40:3586.
- Soback S. 1988. Pharmacokinetics of single doses of cefoxitin given by the intravenous and intramuscular routes to unweaned calves. *J Vet Pharm Ther* 11:155.
- Wraight MD. 2003. A comparative efficacy trial between cefuroxime and cloxacillin as intramammary treatments for clinical mastitis in lactating cows on commercial farms. *NZ Vet J* 51:26.
- Zhao S, et al. 2001. Identification and expression of cephamycinase *bla_{CMY}* genes in *Escherichia coli* and *Salmonella* isolates from food animals and ground meat. *Antimicrob Agents Chemother* 45:3647.
- Zhao S, et al. 2003. Characterization of *Salmonella enterica* serotype Newport isolated from humans and food animals. *J Clin Microbiol* 41:5366.

Group 4 Third-generation Parenteral Cephalosporins: Cefotaxime, Ceftizoxime, Ceftriaxone, Ceftiofur, Latamoxef

Third-generation, group 4, parenteral cephalosporins are distinguished by their high antibacterial activity

and their broad resistance to beta-lactamases; they have particularly good activity against most Enterobacteriaceae. Exceptions include *Enterobacter* and *Serratia*. Streptococci are highly susceptible, staphylococci moderately susceptible, and enterococci are resistant. Latamoxef (moxalactam) is an oxacephem with an oxygen atom replacing the sulfur at the C₁ position of the cephalosporin nucleus. Its wide anti-Enterobacteriaceae activity is similar to that of others in the group but latamoxef is more active against *B. fragilis*, *Citrobacter* spp., and *Enterobacter* spp., and less active against *S. aureus* (Table 8.7). Some *P. aeruginosa* isolates are resistant. When administered, ceftiofur is rapidly metabolized to the active metabolite desfuroylceftiofur. Desfuroylceftiofur is less active than ceftiofur against *Staphylococcus aureus* and *Proteus* spp. Diagnostic laboratories use a ceftiofur disk for susceptibility testing because of the instability of desfuroylceftiofur, so susceptibility testing results for staphylococci and *Proteus* spp. may not be reliable for predicting the efficacy of ceftiofur therapy.

Good Susceptibility ($MIC \leq 2 \mu\text{g/ml}$). Highly active against streptococci, including *Streptococcus suis* (not enterococci). Good activity against many other Gram-positive bacteria (benzyl penicillin sensitive) (Table 8.2, 8.7). Among Gram-negative bacteria, *E. coli*, *Klebsiella* spp., *Proteus* spp. and *Salmonella* spp. are susceptible. Fastidious Gram-negative bacteria (*Actinobacillus* spp., *Haemophilus* spp., *Pasteurella* spp.) including beta-lactamase producers are all highly susceptible. *Clostridium* spp. and *Fusobacterium* spp. are susceptible but *Bacteroides* spp. are often resistant.

Moderately Susceptible ($4 \mu\text{g/ml}$). *S. aureus*; some *Citrobacter* spp., *Enterobacter* spp., some *P. aeruginosa*, and *Serratia* spp. (Table 7.6).

Resistance ($MIC \geq 8 \mu\text{g/ml}$). *Acinetobacter* spp., *Bordetella* spp., some *Enterobacter* spp. and *Serratia* spp., some *P. aeruginosa*, enterococci, and methicillin-resistant *S. aureus*.

Antibiotic Resistance

Transferable resistance to third-generation cephalosporins as a result of AmpC hyperproduction, extended spectrum beta-lactamases, and to a lesser extent beta-lactamase Group 3 metallo-beta-lactamases

Table 8.7. Susceptibility (MIC_{90} , $\mu\text{g/ml}$) of selected animal pathogens to ceftiofur.

Organism	MIC_{90}
Gram-positive aerobes	
<i>Staphylococcus aureus</i>	1
<i>Streptococcus dysgalactiae</i>	≤ 0.004
<i>Streptococcus equi</i>	≤ 0.004
<i>Streptococcus hyicus</i>	1
<i>Streptococcus suis</i>	0.12
<i>Streptococcus uberis</i>	0.03
Gram-negative aerobes	
<i>Actinobacillus pleuropneumoniae</i>	≤ 0.004
<i>Escherichia coli</i>	0.5
<i>Haemophilus parasuis</i>	0.06
<i>Histophilus somni</i>	≤ 0.004
<i>Mannheimia haemolytica</i>	≤ 0.008
<i>Moraxella bovis</i>	0.25
<i>Pasteurella multocida</i>	≤ 0.004
<i>Salmonella</i> spp.	1
Anaerobic bacteria	
<i>Bacteroides fragilis</i>	≥ 16
<i>Bacteroides</i> spp.	4
<i>Fusobacterium necrophorum</i>	≤ 0.06
<i>Peptostreptococcus anaerobius</i>	0.12

(Table 9.1), has been discussed earlier and is an increasing threat to the continued use of these cephalosporins in animals. In recent years, multidrug resistance plasmids carrying the *bla*_{CMY2} encoding resistance to ceftiofur and ceftriaxone have been identified in *Salmonella enterica* serovars Newport and Typhimurium, among others, and is often found in strains with concomitant resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (Doublet et al. 2004). The *cmv-2* gene appears to have been mobilized into different plasmid backbones that have spread through conjugation (Carattoli et al., 2002).

In human medicine, the breakpoint for resistance is $64 \mu\text{g/ml}$, leading to confusion between resistance reported for animal isolates and human isolates. For example, in the Canadian Integrated Program for Antimicrobial Resistance Surveillance report for 2003 data (CIPARS 2005), ceftiofur resistance (breakpoint $\geq 8 \mu\text{g/ml}$) was particularly high in *Salmonella* isolated from chicken in Québec but not as high for human *Salmonella* isolates tested for ceftriaxone (breakpoint $\geq 64 \mu\text{g/ml}$); when the same $8 \mu\text{g/ml}$ breakpoint was applied to both drugs, the percentage

resistant was the same. The high prevalence of ceftiofur resistance in *Salmonella* isolated from Québec was attributed to the practice of *in ovo* injection with ceftiofur to control colibacillosis.

Pharmacokinetic Properties

Third-generation, group 4, parenteral cephalosporins are not absorbed after oral administration but are rapidly well-absorbed after IM or SC administration, giving peak serum levels in 0.5–1 hour. While data are often lacking, the half-life is about 1 hour following IV infection. In cattle, the half-life of ceftiofur is about 2.5 hours. By contrast, the half-lives for many of these cephalosporins are 1–2 hours for humans. Ceftriaxone is extensively protein-bound and has a half-life of 8 hours, giving it the potential for once-daily dosing. The half-life of latamoxef in humans is about 2 hours. Ceftiofur is rapidly metabolized by plasma esterases to the active metabolite desfuroylceftiofur. Both the parent compound and the active metabolite are highly protein bound, resulting in long elimination half-lives (3–12 hours) and allowing once daily dosing.

Distribution into tissues in extracellular fluid is widespread, but passage across membranes or physiological barriers is poor. Meningeal inflammation significantly enhances otherwise poor CSF penetration so these cephalosporins are drugs of choice for bacterial meningitis caused by Enterobacteriaceae. Cefotaxime is metabolized in the body to the less active desacetyl-cefotaxime. Excretion is largely through the urinary tract, with cefotaxime being excreted through tubular mechanisms and the others through glomerular filtration. Probenecid administration delays tubular excretion. Biliary elimination also occurs, notably for ceftriaxone and latamoxef. These drugs should therefore be avoided in species with expanded large intestines. Ceftriaxone has a long elimination half-life, giving this drug the advantage of twice-daily dosing.

A crystalline free acid formulation of ceftiofur administered as a single subcutaneous injection into the ear of cattle at 6.6 mg/kg is slowly absorbed and gives plasma concentrations exceeding the MIC of common respiratory tract bacterial pathogens for about six days (Hibbard et al., 2002). This formulation administered intramuscularly in swine also has a long half-life, with plasma concentrations after intramuscular injection of 5 mg/kg exceeding the MIC of common respiratory tract pathogens for about five days.

Drug Interactions

Group 4 cephalosporins are synergistic with aminoglycosides, with which they should often be combined in the treatment of febrile illness in neutropenic human patients.

Toxicities and Side Effects

Toxicities and side effects are similar to those described for group 1–3 cephalosporins, but the nephrotoxic potential is low. Cefmenoxime in humans produces alcohol intolerance by blocking liver acetaldehyde dehydrogenase and a coagulopathy associated with hypoprothrombinemia, which is reversible by vitamin K. Clinically important bleeding disorders caused by hypoprothrombinemia or disorders of platelet function are more common with latamoxef than with any other cephalosporin in human patients (about 20%), so that this drug is not generally recommended for clinical use. Vitamin K prophylaxis is suggested if the drug is used. Anemia and thrombocytopenia were seen in dogs given high doses of ceftiofur for prolonged periods of time.

Because of the broad antibacterial activity of these cephalosporins, gastrointestinal disturbances and superinfection by resistant microorganisms, including yeasts, might be anticipated. In human medicine, there is a strong association between groups 4 and 6 cephalosporin use and *C. difficile* diarrhea. In horses, IM administration has occasionally been associated with gastrointestinal disturbance, including severe colitis. Gastrointestinal disturbances were noted in four of six mares administered ceftriaxone IV (Gardner and Aucoin, 1994), probably because of its biliary excretion, so this drug should be used cautiously if at all in horses. Cutaneous drug reaction to ceftiofur, characterized by hair loss and pruritus, has been described in a cow (Tyler et al., 1998). Intra-arterial injection of ceftiofur crystalline free acid may be fatal.

Administration and Dosage

Recommended IM dosages, which in some cases are empirical, are shown in Table 8.7. To some extent, dosage can be tailored to the susceptibility of the organism, with the aim being to maintain drug concentrations \geq MIC throughout the majority of the dosing interval. For example, dosage of ceftiofur for highly susceptible organisms associated with respiratory disease is usually 1.1–2.2 mg/kg every 24 hours, but for *E. coli* infections caused by susceptible organisms the

dose might be as high as 2.2–4.4 mg/kg every 12 hours. Ceftriaxone has the advantage that dosage is twice daily whereas dosage of other group 4 cephalosporins (other than ceftiofur) is usually every 8 hours; every 12 hour administration of cefotaxime was found to be as effective as every 8 hour administration in the treatment of mild to moderate non-CNS infections in people (Brogden and Spencer, 1997).

Clinical Applications

Because of expense, the availability of cheaper alternatives, and the potential to select for resistant bacteria, third-generation group 4 cephalosporins should be reserved for serious, life-threatening infections caused by Gram-negative bacteria, especially Enterobacteriaceae. These cephalosporins are drugs of choice in meningitis caused by *E. coli* or *Klebsiella* spp. They are recommended, in combination with an aminoglycoside, in severe infections caused by multiresistant bacteria in compromised hosts, such as neutropenic hosts. These drugs have potential applications in septicemia, serious bone and joint infections, some lower respiratory tract infections, intra-abdominal infections caused by Enterobacteriaceae, and some soft tissue infections where cheaper alternatives are not available. There is increasing interest in their value in treating systemic complications of human salmonellosis (bacteremia, meningitis, osteomyelitis).

The poor activity of some of these cephalosporins against Gram-negative anaerobes is a drawback; ceftiofur, however, has good activity against anaerobes. Although not well documented, they have a tendency to select for *Clostridium difficile* infections in species such as horses and swine. Despite the recommendations to reserve these drugs for serious infections, and only where susceptibility testing indicates that alternatives are not available, there is an increasing tendency to use these drugs as first choice in animals.

Cattle, Sheep, and Goats

Ceftiofur is used extensively for the treatment of acute, undifferentiated bovine pneumonia with the advantage of a low recommended dose (1.1–2.2 mg/kg, every 24 hours). The sodium ceftiofur formulation is given IM and has a zero withdrawal time in meat and milk. The more stable ceftiofur hydrochloride formulation is given IM or SC and has a 2-day meat withdrawal time in the United States and a 3-day meat withdrawal time in Canada, but a zero milk with-

drawal time in both countries. Treatment is for 3–5 days and has proved as effective as treatment with sulbactam-ampicillin or potentiated sulfonamides. A crystalline free acid suspension formulation has been recently approved for subcutaneous injection in the ears of cattle. This slow-release formulation acts as a constant infusion and maintains plasma concentrations of ceftiofur above the MIC of bovine respiratory disease pathogens for 150 hours.

Ceftiofur administration at 3 mg/kg every 12 hours was inadequate for the parenteral treatment of mastitis caused by *E. coli* and 2.2 mg/kg every 24 hours failed to remove *S. agalactiae* infections, since udder distribution is poor because of ceftiofur's high plasma protein binding (Erskine et al., 1995, 1996). Treatment of severe coliform mastitis with ceftiofur was, however, shown to reduce death or culling (Erskine et al., 2002). Dosage at 1 mg/kg IM was effective in the treatment of footrot in feedlot cattle (Morck et al., 1998). Ceftiofur has also been used experimentally at the extra-label dose of 5 mg/kg q24 hours in the treatment of *Salmonella* infection of calves (Fecteau et al., 2003). Multiple treatments with ceftiofur have been used to eliminate *Leptospira* from the kidneys of cattle, although tetracycline and tilmicin were equally effective and may be preferred as less likely to induce important resistance in "bystander" bacteria (Alt et al., 2001). As mentioned, an unusual new crystalline free acid formulation of ceftiofur administered into the ear at 6.6 mg/kg gives plasma concentrations exceeding the MIC of respiratory tract pathogens (*H. somni*, *M. haemolytica*, *P. multocida*) for over five days.

Horses

Ceftiofur is approved in horses for treating bacterial infections caused by *Streptococcus zooepidemicus* (Table 8.7). At 2.2 mg/kg IM every 24 hours, it was as effective as ampicillin in the treatment of respiratory infections in adult horses (Folz et al., 1992). Doses up to 11 mg/kg/day for 30 days were well tolerated. Intramuscular rather than oral administration is a drawback. The drug has potential application for treatment of septicemia in foals, perhaps combined with an aminoglycoside. It has been used successfully to treat pleuritis and peritonitis caused by susceptible organisms. Cefotaxime has been used effectively in the treatment of neonatal septicemia and meningitis caused by *Acinetobacter* spp., *Enterobacter* spp., and *P. aeruginosa*. Ceftriaxone may be particularly suitable for the

treatment of meningitis in foals because it crosses the healthy blood-cerebrospinal fluid barrier. A dosage suggested for Gram-negative bacterial meningitis was 25 mg/kg every 12 hours (Ringger et al., 1998). This drug should, however, be used with caution in adult horses because of its hepatic excretion.

Swine

Ceftiofur is available for use in swine in the treatment of respiratory or systemic infections caused by susceptible bacteria such as *P. multocida*, beta-lactamase producing *Actinobacillus* spp., *Haemophilus parasuis*, and *Streptococcus suis*. A new crystalline free acid formulation of ceftiofur administered aurally at 5 mg/kg gives plasma concentrations exceeding the MIC of respiratory tract pathogens (*A. pleuropneumoniae*, *H. parasuis*, *P. multocida*, *S. suis*) for over five days. Ceftiofur has been used in the control of *Salmonella choleraesuis* infections. It also has application for IM administration in the treatment of neonatal colibacillosis. The practice of routine injection of neonatal pigs with ceftiofur may, however, predispose them to infection with *Clostridium difficile*, which has emerged as a significant problem in some swine farms in recent years. Narrower spectrum drugs are often effective and should be preferred for the clinical applications outlined above. Multidrug (including ceftiofur [*cmv*-2] and fluoroquinolone) resistant *S. choleraesuis* infections from swine have been described in humans (Yan et al., 2005).

Dogs and Cats

Group 4 cephalosporins might be useful in the treatment of urinary tract infections caused by otherwise resistant bacteria. Ceftiofur is labeled for treatment of urinary tract infections in dogs, but as it must be administered parenterally, it is rarely used.

Poultry

Ceftiofur is administered IM or *in ovo* to chicks and turkey poults for the control of *E. coli* infections. The emergence of resistant *Salmonella* in broilers as a result of egg injection has been noted earlier.

Bibliography

Alt DP, et al. 2001. Evaluation of antibiotics for treatment of cattle infected with *Leptospira borgpetersenii* serovar hardjo. J Am Vet Med Assoc 219:636.

- Brown SA, et al. 1995. Plasma and urine disposition and dose proportionality of ceftiofur and metabolites in dogs after subcutaneous administration of ceftiofur sodium. J Vet Pharm Ther 18:363.
- Canadian Integrated Program for Antimicrobial Resistance Surveillance, 2003. Government of Canada, Public Health Agency of Canada, 2005. 110 pp.
- Carattoli A, et al. 2002. Characterization of plasmids carrying CMY-2 from expanded cephalosporin-resistant *Salmonella* strains isolated in the United States between 1996 and 1998. Antimicrob Agents Chemother 46:1269.
- Doublet B, et al. 2004. Plasmid-mediated florfenicol and ceftriaxone resistance encoded by the *florR* and *bla_{CMY2}* genes in *Salmonella enterica* serovars Typhimurium and Newport isolated in the United States. FEMS Microbiol Lett 233:301.
- Erskine RJ, et al. 1995. Ceftiofur distribution in serum and milk from clinically normal cows and cows with experimental *Escherichia coli*-induced mastitis. Am J Vet Res 56:481.
- Erskine RJ, et al. 1996. Intramuscular administration of ceftiofur sodium versus intramammary infusion of penicillin/novobiocin for treatment of *Streptococcus agalactiae* mastitis in dairy cows. J Am Vet Med Assoc 208:258.
- Erskine RJ, et al. 2002. Efficacy of systemic ceftiofur as a therapy for severe clinical mastitis in dairy cattle. J Dairy Sci 85:2571.
- Fecteau ME, et al. 2003. Efficacy of ceftiofur for treatment of experimental salmonellosis in neonatal calves. Am J Vet Res 64:918.
- Folz SD, et al. Treatment of respiratory infections in horses with ceftiofur sodium. Equine Vet J 24:300.
- Gardner SY, Aucoin DP. 1994. Pharmacokinetics of ceftriaxone in mares. J Vet Pharm Ther 17:155.
- Hibbard B, et al. 2002. Dose determination and confirmation of ceftiofur crystalline-free acid administered in the posterior aspect of the ear for control and treatment of bovine respiratory disease. Vet Therap 3:22.
- Hibbard B, et al. 2003. Efficacy of ceftiofur sodium for the control of mortality in neonatal pigs orally inoculated with K88⁺ (F4⁺) enterotoxigenic *Escherichia coli*. J Swine Hlth Prod 11:7.
- McElroy D, et al. 1986. Pharmacokinetics of cefotaxime in the domestic cat. Am J Vet Res 47: 86.
- Meeuwse DM, et al. Effectiveness of a single intramuscular dose of ceftiofur hydrochloride for the treatment of naturally occurring bacterial swine respiratory disease. J Swine Hlth Prod 10:113.
- Meyer JC, et al. 1992. Pharmacokinetics of ceftiofur sodium in neonatal foals after intramuscular injection. Equine Vet J 24:485.
- Morck DW, et al. 1998. Comparison of ceftiofur sodium and oxytetracycline for treatment of acute interdigital phlegmon (foot rot) in feedlot cattle. J Am Vet Med Assoc 212:254.
- Ringger NC, et al. 1998. Pharmacokinetics of ceftriaxone in neonatal foals. Equine Vet J 30:163.
- Salmon SA, et al. 1996. In vitro activity of ceftiofur and its primary metabolite, desfurioylceftiofur, against organisms of veterinary importance. J Vet Diagn Invest 8:332.

- Samitz EM, et al. 1996. In vitro susceptibilities of selected obligate anaerobic bacteria obtained from bovine and equine sources to ceftiofur. *J Vet Diagn Invest* 8:121.
- Shryock TR, et al. 1998. Antimicrobial susceptibility of *Moraxella bovis*. *Vet Microbiol* 61:305.
- Soback S, et al. 1989. Pharmacokinetics of ceftiofur administered intravenously and intramuscularly to lactating cows. *Isr J Vet Med* 45:119.
- Soback S. 1989. Pharmacokinetics of single-dose administration of moxalactam in unweaned calves. *Am J Vet Res* 50:498.
- Tell L, et al. 1998. Pharmacokinetics of ceftiofur sodium in exotic and domestic avian species. *J Vet Pharm Ther* 21:85.
- Tyler JW, et al. 1998. Probable ceftiofur-induced cutaneous drug reaction in a cow. *Can Vet J* 39:296.
- Yan J-J, et al. 2005. Cephalosporin and ciprofloxacin resistance in *Salmonella*, Taiwan. *Emerg Infect Dis* 11:947.

Group 5 Third-generation Oral cephalosporins: Cefetamet, Cefixime, Cefpodoxime

Third-generation, group 5, oral cephalosporins are highly active cephalosporins resistant to many beta-lactamases and available for oral administration. Cefixime is structurally related to cefotaxime and cefizoxime and shares their antibacterial activity. Cefetamet pivoxil is a prodrug hydrolysed to the active cefetamet. It largely shares the antibacterial spectrum of cefixime and other group 4 parenteral cephalosporins. Cefpodoxime proxetil is also a prodrug that is absorbed from and de-esterified in the gastrointestinal tract to release the active metabolite cefpodoxime.

Antimicrobial Activity

Antimicrobial activity is similar to that of Group 4, third-generation parenteral cephalosporins. Among Gram-positive aerobes, third-generation oral cephalosporins are relatively inactive against *S. aureus* (MIC₉₀ canine *S. aureus* = 2 µg/ml), active against pyogenic streptococci but inactive against enterococci. They have good activity against many other benzylpenicillin sensitive Gram-positive bacteria (Tables 8.2, 8.7). They have broad activity against Enterobacteriaceae, though this may exclude some *Citrobacter* and *Enterobacter*. *Pseudomonas* are resistant. Fastidious Gram-negative bacteria (*Actinobacillus* spp., *Haemophilus* spp., *Pasteurella* spp.), including beta-lactamase producers, are all highly susceptible. Among human pathogens, third-generation oral cephalosporins are active against beta-lactamase producing *Haemophilus*

spp. but inactive against penicillin-resistant *Streptococcus pneumoniae*. *Clostridium* spp. and *Fusobacterium* spp. are susceptible but *Bacteroides* spp. are often resistant. Proposed breakpoints for cefpodoxime for use in dogs are: Susceptible ≤ 2 µg/ml, Intermediate 4 µg/ml, and Resistant ≥ 8 µg/ml.

Antibiotic Resistance

Antimicrobial activity is similar to that of group 4, third-generation parenteral cephalosporins.

Pharmacokinetic Properties

The pharmacokinetic properties of group 5 cephalosporins are typical of those of beta-lactams generally. Cefpodoxime has a relatively long half-life in dogs (5.6 hours), so that plasma concentrations exceed 1 µg/ml for about 24 hours after a dose of 10 mg/kg.

Drug Interactions

Group 5 cephalosporins are synergistic with aminoglycosides, with which they are often combined in the treatment of febrile illness in neutropenic human patients.

Toxicities and Side Effects

Adverse effects of group 5 cephalosporins in humans involve mainly gastrointestinal disturbance (diarrhea, nausea, vomiting), which occur in about 10% of patients. Similar effects might be anticipated in animals. Like all broad-spectrum antimicrobial drugs, they should not be administered to herbivores with expanded large intestines. Cefpodoxime administered orally to dogs has been associated with no adverse effects.

Administration and Dosage

Dosage recommendations for cefetamet in children are 8 mg/kg every 24 hours or 4 mg/kg q12 hours. A suggested dosage for dogs is in the same range (Lavy et al., 1995) and for preruminant calves 10 mg/kg q24 hours (Ziv et al., 1995). Cefixime's long elimination half-life allows once-daily administration in people. Dosage recommended for cefetamet in children is 20 mg/kg q12 hours. Cefpodoxime has been approved in the United States for skin infections in dogs at 5–10 mg/kg once daily. The higher dose is preferable for *S. aureus* infections. A suggested dosage of cefpodoxime in foals was 10 mg/kg every 6–12 hours (Carrillo et al., 2005).

Clinical Applications

Cefetamet is used in the treatment of upper respiratory and urinary tract infections in people. Cefixime is used in people for the same purposes as cefetamet and has been advocated as an oral "follow-up" to a group 4 parenteral cephalosporin. Cefpodoxime has been approved for use in dogs in the United States for skin infections (wounds and abscesses) caused by susceptible organisms. Second and third generation cephalosporins are not first-choice antimicrobial agents in animals and should be reserved for use where susceptibility testing indicates that alternatives are not available.

Bibliography

- Bryson HM, Brogden RN. 1993. Cefetamet pivoxil. *Drugs* 45:589.
- Carillo NA, et al. 2005. Disposition of orally administered cefpodoxime proxetil in foals and adult horses and minimum inhibitory concentration of the drug against common bacterial pathogens of horses. *Am J Vet Res* 66:30.
- Lavy E, et al. 1995. Clinical pharmacologic aspects of cefixime in dogs. *Am J Vet Res* 56:633.
- Markham A, Brogden RN. 1995. Cefixime. *Drugs* 49:1007.
- Pfizer Animal Health. Product Monograph Simplicef® (cefpodoxime proxetil tablets). New York: Pfizer, Inc. http://www.simplicef.com/images/simplicef_pre_info.pdf.
- Ziv G, et al. 1995. Clinical pharmacology of cefixime in unweaned calves. *J Vet Pharm Ther* 18:94.

Group 6 Antipseudomonal Parenteral Cephalosporins: Cefoperazone, Cefsulodin, and Ceftazidime

Antipseudomonal, group 6, parenteral cephalosporins are distinguished by high activity against *P. aeruginosa*. Cefsulodin has otherwise a very narrow spectrum of activity. Ceftazidime and cefoperazone have a spectrum of activity almost identical to group 4 cephalosporins but with approximately ten and three times greater activity against *P. aeruginosa*, respectively (Table 8.2). Resistance to ceftazidime is rare in *P. aeruginosa*. The group 6 drugs are otherwise slightly less active than group 4 drugs against most organisms. Antipseudomonal cephalosporins are synergistic with aminoglycosides, with which they are often combined in the treatment of *P. aeruginosa* infections in neutropenic human patients. Resistance, because of AmpC beta-lactamases, has been described in *Entero-*

Table 8.8. Empirical IM dosage of Group 6 antipseudomonal parenteral cephalosporins.

Species	Drug	Dose (mg/kg)	Interval (h)
Dog, cat	Cefoperazone	20	6-8
	Ceftazidime	25-50	8-12
Cattle	Cefoperazone	30	6-8
	Ceftazidime	20-40	12-24
Horse (caution)	Cefoperazone	30	6-8
	Ceftazidime	25-50	8-12

bacter, *Citrobacter*, *Serratia* and other genera of the Enterobacteriaceae, and through ceftazidime-specific PER type extended-spectrum beta-lactamases (Table 9.1).

Pharmacokinetic properties are similar to those described for other parenteral cephalosporins. One exception is the largely hepatic elimination of cefoperazone, which leads to gastrointestinal disturbance in humans. Cefoperazone, but not ceftazidime, elimination in urine is reduced by probenecid. There has been little study of these drugs' pharmacokinetic properties in animals. In calves, half-life is about 2 hours (Soback and Ziv, 1989a, 1989b).

Toxicities and side effects are the same as for other cephalosporins generally. Cefoperazone is likely contraindicated in those herbivore species with an expanded large bowel.

Empirical dosages are shown in Table 8.8.

These drugs are largely reserved in human medicine for *P. aeruginosa* and other Gram-negative septicemias in neutropenic human patients, in which efficacy is considerably enhanced by combination with an aminoglycoside. Cephalosporins have slow bactericidal activity compared to aminoglycosides. Subcutaneous injection of 30 mg/kg every 4 hours or constant IV infusion of 4.1 mg/kg/hour produced serum concentrations exceeding the MIC of canine clinical isolates of *P. aeruginosa* (Moore et al., 2000).

Cefoperazone has been used by the intramammary route as a broad-spectrum antibiotic for the treatment of bovine mastitis (Wilson et al., 1986). The advantage claimed is that a single infusion of 250 mg in an oil base gives milk concentrations \geq MIC of common pathogens for up to 48 hours but, because of systemic absorption, milk concentrations are very low by the fifth milking, which reduces the cost of discarded milk. The drug may have particular advantage in the treat-

ment of coliform mastitis. The rapid development of resistance by bacteria possessing inducible, chromosomal cephalosporinases must be anticipated, but has not yet been documented in veterinary medicine.

Bibliography

- Moore KW, et al. 2000. Pharmacokinetics of ceftazidime in dogs following subcutaneous administration and continuous infection and the association with in vitro susceptibility of *Pseudomonas aeruginosa*. *Am J Vet Res* 61:1204.
- Rains CP, et al. 1995. Ceftazidime. *Drugs* 49:577.
- Rice LB, et al. 1990. Outbreak of ceftazidime resistance caused by extended-spectrum beta-lactamases at a Massachusetts chronic-case facility. *Antimicrob Agents Chemother* 34:2193.
- Soback S, Ziv G. 1989a. Pharmacokinetics of single doses of cefoperazone given by the intravenous and intramuscular routes to unweaned calves. *Res Vet Sci* 47:158.
- Soback S, Ziv G. 1989b. Pharmacokinetics of ceftazidime given alone and in combination with probenecid to unweaned calves. *Am J Vet Res* 50:1566.
- Wilson CD, et al. 1986. Field trials with cefoperazone in the treatment of bovine clinical mastitis. *Vet Rec* 118:17.

Group 7 Fourth-generation Parenteral Cephalosporins: Cefepime, Cefpirome, Cequinome

Although sometimes considered as part of the group 6 parenteral cephalosporins, the "fourth-generation", group 7, parenteral cephalosporins have high activity against Enterobacteriaceae, moderate activity against *P. aeruginosa*, and enhanced activity against staphylococci. They are stable to hydrolysis by many plasmid- or chromosomally-mediated beta-lactamases and are poor inducers of group 1 beta-lactamases.

Antimicrobial Activity

Cefepime is an enhanced-potency, extended-spectrum cephalosporin. Its zwitterionic nature gives it rapid ability to penetrate through the porins of Gram-negative bacteria to the cell membrane. Both cefepime and cefpirome have higher affinity for essential PBPs and greater resistance to hydrolysis by beta-lactamases than other cephalosporins. In particular, they are resistant to, and a poor inducer of, group 1 beta-lactamases. There are no reports of activity against specific animal pathogens, however.

Good susceptibility ($MIC \leq 8 \mu\text{g/ml}$): Methicillin-susceptible *Staphylococcus* spp., *Streptococcus* spp., Enterobacteriaceae including *Citrobacter* spp., *Entero-*

Table 8.9. Antimicrobial activity (MIC_{90} , $\mu\text{g/ml}$) of cefepime and cefpirome.

Organism	Cefepime	Cefpirome
<i>Staphylococcus aureus</i> (methicillin-sensitive)	2	0.5
<i>Streptococcus agalactiae</i>	0.12	0.06
<i>Enterococcus faecalis</i>	16	4
<i>Escherichia coli</i>	0.12	0.12
<i>Proteus mirabilis</i>	0.06	0.06
<i>Pseudomonas aeruginosa</i>	4	8
<i>Acinetobacter</i> spp.	8	4

bacter spp. *E. coli*, and *Serratia* resistant to group 4 cephalosporins; *P. aeruginosa*, including isolates resistant to group 6 cephalosporins; beta-lactamase producing *Haemophilus* spp.; *C. perfringens*, *Peptostreptococcus* spp. (Table 8.9).

Resistance ($MIC \geq 32 \mu\text{g/ml}$): *Enterococcus* spp., *L. monocytogenes*, *Bacteroides* spp., *C. difficile*.

Pharmacokinetic Properties

Pharmacokinetic properties of these parenterally administered cephalosporins are typical of those of other parenteral cephalosporins generally. Most are excreted through the urine.

Drug Interactions

Combination of cefepime with aztreonam is synergistic against *P. aeruginosa* with derepressed cephalosporinases, since aztreonam protects cefepime against these enzymes in the extracellular environment (Lister et al., 1998).

Toxicities and Adverse Effects

Toxicities and adverse effects in people are those of cephalosporins generally, with the major effect being gastrointestinal disturbance. Treatment was withdrawn in about 5% of patients treated with cefpirome and 1–3% of patients treated with cefepime because of adverse effects. Gastrointestinal effects must be anticipated if these drugs are used in animals, and have been observed in horses administered cefepime by the oral or IM route (Guglick et al., 1998).

Administration and Dosage

These drugs are administered IV or IM twice daily to human patients; dosage can to some extent be tailored

to the nature and severity of the infection. In horses, a dosage recommendation was 2.2 mg/kg every 8 hours (Guglick et al., 1998). This is a very low dosage based on extrapolation from the empirical dose of 50 mg/kg every 8 hours in children. By contrast, an IV dose estimated for treatment of susceptible bacteria in neonatal foals was 11 mg/kg every 8 hours and for dogs, 40 mg/kg every 6 hours (Gardner and Papich, 2001). In cattle and swine, a dose of 1–2 mg/kg every 24 hours IM is used.

Clinical Applications

Fourth-generation cephalosporins are used in human medicine in the treatment of nosocomial or community acquired lower respiratory disease, bacterial meningitis, urinary tract infections and uncomplicated skin or skin related infections. They have shown no advantage in clinical trials comparing them to cefotaxime or ceftazidime in treatment of infections in people. These drugs are valuable extended-spectrum cephalosporins for the treatment of serious human infections. Cefquinome is used in Europe and Japan to treat bovine respiratory disease and, by intramammary or IM administration, in the treatment of coliform and other bacterial mastitis. In cattle and swine, its efficacy in field studies has been similar or slightly

superior to that of ceftiofur (Funaki et al., 2001; Lang et al., 2003). Second and third generation cephalosporins are not first-choice antimicrobial agents in animals, but rather should be reserved for use where susceptibility testing indicates that alternatives are not available.

Bibliography

- Barradell LB, Bryson HM. 1994. Cefepime. *Drugs* 47:471.
- Funaki H, et al. 2001. Therapeutic efficacy of cefquinome against acute respiratory disease in Holstein steers. *J Jpn Vet Med Assoc* 54:451.
- Gardner SY, Papich MG. 2001. Comparison of cefepime pharmacokinetics in neonatal foals and adult dogs. *J Vet Pharm Therap* 24:187.
- Guglick MA, et al. 1998. Pharmacokinetics of cefepime and comparison with those of ceftiofur in horses. *Am J Vet Res* 59:458.
- Lang I, et al. 2003. A field study of cefquinome for the treatment of pigs with respiratory disease. *Rev Med Vet* 153:575.
- Lister PD, et al. 1998. Cefepime-aztreonam: a unique double β -lactam combination for *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 42:1610.
- Sanders CC. 1993. Cefepime: the next generation? *Clin Infect Dis* 17:369.
- Wiseman LR, Lamb HM. 1997. Cefepime. *Drugs* 54:117.

Other Beta-lactam Antibiotics: Beta-lactamase Inhibitors, Carbapenems, and Monobactams

John F. Prescott

The continuing development of beta-lactam antibiotics by changes of atoms within the basic beta-lactam ring or its attachment to the thiazolidine ring has produced compounds with significantly different activity from penam penicillins and the cephalosporins and cephamycins. Carbapenem and monobactam class antibiotics (Figure 7.2) have been introduced into human medicine, but none have been approved for use in veterinary medicine. By contrast, some beta-lactamase inhibitors (clavulanic acid, sulbactam) have been successfully introduced into veterinary medicine in combination with aminobenzylpenicillins, producing broad-spectrum antibacterial drugs which overcome the acquired resistance that limits use of the older extended-spectrum penicillins.

Beta-lactamase Inhibitors: Clavulanic Acid, Sulbactam, and Tazobactam

Introduction

Beta-lactamase production is a major factor in constitutive or acquired resistance of bacteria to beta-lactam antibiotics. The clinical importance of beta-lactamases is associated with the rapid ability of plasmid-mediated resistance to spread through bacterial populations. Such resistance has considerably reduced the value of very important drugs, such as amoxicillin. Three beta-lactamase inhibitors, clavulanic acid, sulbactam and tazobactam (Figure 9.1), have considerably enhanced the activity of penicillins against bacteria with acquired plasmid-mediated resistance. Although possessing weak antibacterial activity on their own, their irreversible binding to susceptible

beta-lactamases (Table 9.1) allows the active beta-lactam antibiotic, with which they are combined, to bind to the penicillin binding proteins (PBPs) and kill the bacterial pathogen. Antibiotics combined for clinical use with clavulanic acid or sulbactam, which both have similar spectrum of beta-lactamases-inhibiting activities, include amoxicillin, ampicillin, and ticarcillin. Clavulanic acid and sulbactam are synergistic with penicillins and cephalosporins that are readily hydrolyzed by plasmid-mediated beta-lactamases, including benzyl- and aminobenzylpenicillins and third-generation cephalosporins. Introduction of clavulanic acid and sulbactam has been a significant advance in antimicrobial therapy of infections in animals. The beta-lactamase inhibitors should be used with caution in herbivores with expanded large intestines because of potential for disrupting normal flora resulting in diarrhea.

Beta-lactamases: Classification

Beta-lactamases are enzymes which degrade beta-lactam drugs by opening the beta-lactam ring. As described in Chapter 8, there has been a remarkable evolution of these enzymes in response to antimicrobial selection. The encoding genes have disseminated widely through Gram-negative bacterial populations via plasmids and transposons. The beta-lactamases of clinically important pathogens have been studied in exquisite detail. They consist of a wide variety of related proteins, hundreds of which have been fully characterized. They may be chromosomally mediated (inducible or constitutive) or plasmid-mediated. Beta-lactamases of Gram-positive bacteria may be exported extracellularly, whereas beta-lactamases of Gram-

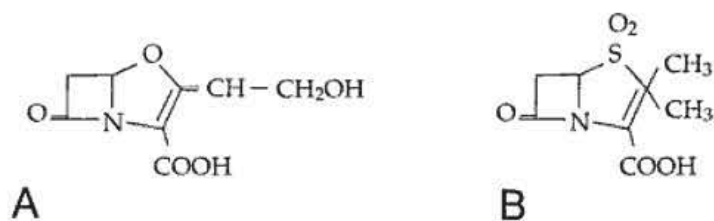


Figure 9.1. Structural formulas of clavulanic acid (A) and sulbactam (B).

negative bacteria are normally found in the periplasmic space but may be found extracellularly when the bacterium lyses (Figures 7.3, 7.4).

Classification is based on a combination of molecular characterization (nucleotide, amino acid sequence) and functional characterization (substrate, inhibition profile) (Table 9.1). The functional groups described by Bush (2001) are identified particularly by their inhibition by clavulanic acid and EDTA as well as according to substrate hydrolysis profiles (e.g., benzyl penicillin, ceftazidime, cefotaxime, imipenem). Although there is general correlation with molecular based typing approaches, a functional approach to classification is preferred because very fine differences in molecular character may cause dramatic differences in function. For example, a mutation producing a single nucleotide change can convert a TEM-1 to a TEM-12 beta-lactamase, and a second single nucleotide change can convert this TEM-12 enzyme into a TEM-26 beta-lactamase, with marked effects on hydrolysis of ceftazidime.

The genes for beta-lactamases are found in the chromosome or on plasmids, and may be moved from these sites by transposons. Transfer of some of these genes has been widespread within and between species, genera, and families. The evolution of beta-lactamases has occurred at a dramatic rate among bacteria, probably in response to selection by the extensive use of broad-spectrum beta-lactam antibiotics. Plasmid-mediated beta-lactamases are centrally important in beta-lactamase resistance. For example, plasmid-mediated TEM-1 beta-lactamase, which encodes ampicillin resistance, has become widespread in *E. coli*. More recently, plasmid-mediated extended-spectrum beta-lactamases (subgroup 2be) have emerged among Enterobacteriaceae, although these remain sensitive to ceftazidime and imipenem, and usually also to the beta-lactamase inhibitors clavulanic acid and tazobactam. However, some TEM variants resistant to beta-lactamase inhibitors have been described (Table 9.1).

All Gram-negative bacteria produce beta-lactamases, usually functional group 1, from genes located on their chromosomes. In some genera (e.g., *Acinetobacter*, *Citrobacter*, *Enterobacter*, *Serratia*), as described in Chapter 8 under *Resistance to Cephalosporins*, these AmpC hyperproducers are inducible, producing high concentrations of enzyme that overwhelm local concentrations of beta-lactamase inhibitors. In some cases, mutants with derepressed inducible beta-lactamases have emerged among these bacteria that are now resistant to the beta-lactams. More seriously, as described in Chapter 8, AmpC hyperproduction may be encoded by high copy number plasmids (CMY2, FOX, MIR, MOX). The dissemination of CMY2 AmpC beta-lactamase plasmids among *E. coli* and *Salmonella* is a particular current concern.

Group 3 beta-lactamases are metalloenzymes which hydrolyse most beta-lactams and resist beta-lactamase inhibitors. Genes for these enzymes have been identified on plasmids among opportunist bacteria isolated from human patients.

The most rapidly expanding family of beta-lactamases is the functional subgroup 2be, the extended-spectrum beta-lactamases (ESBL). Identification of ESBLs can be problematic. In the testing procedure recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2000), an MIC ≥ 1 $\mu\text{g/ml}$ for third-generation cephalosporins is followed by testing the MIC for ceftazidime or cefotaxime in the presence of 4 $\mu\text{g/ml}$ clavulanic acid, which should reduce MIC by eightfold (i.e., three two-fold dilutions). A problem is that the sensitivity and specificity of the test varies with the cephalosporin (Bradford, 2001), so that this method may underestimate resistance by not detecting ESBLs at the first level of the test. Another problem is that detection of ESBL-producing bacteria increases with the inoculum, and the usual bacterial inoculum used in susceptibility testing underestimates the presence of ESBLs. This has in vivo significance (Rice et al., 1991). Other systems use a susceptibility disk containing amoxicillin-

Table 9.1. Functional and molecular characteristics of the major groups of beta-lactamases

Functional group	Major subgroups	Molecular class	Attributes of beta-lactamases in functional group	Inhibited by clavulanic acid	Estimated no. of enzymes ^a (Year 2000)
1		C	Often chromosomal enzymes in Gram-negative bacteria. Confer resistance to all classes of beta-lactams, except carbapenems. Not inhibited by clavulanic acid. Plasmid-encoded include LAT, MIR, ACT, FOX, CMY family beta-lactamases	—	51
2		A, D	Most enzymes responsive to inhibition by clavulanic acid (unless noted)		256
	2a	A	Staphylococcal and enterococcal penicillinases included. High resistance to penicillins	+	23
	2b	A	Broad-spectrum beta-lactamases, including TEM-1 and SHV-1, primarily Gram-negative bacteria	+	16
	2be	A	Extended-spectrum beta-lactamases conferring resistance to oxyimino-cephalosporins and monobactams (CTX-M, PER, SHV, some OXA, TEM, VEB)	+	119
	2br	A	Inhibitor-resistant TEM (IRT) beta-lactamases; one inhibitor-resistant SHV-derived enzyme	±	24
	2c	A	Carbenicillin-hydrolyzing enzymes	+	19
	2d	D	Cloxacillin-hydrolyzing enzymes; modestly inhibited by clavulanic acid (OXA family)	±	31
	2e	A	Cephalosporinases	+	20
	2f	A	Carbapenem-hydrolyzing enzymes with active site serine	+	4
3	3a, 3b, 3c	B	Metallo-beta-lactamases conferring resistance to carbapenems and all beta-lactam classes except monobactams (IMP, SPM, VIM). Not inhibited by clavulanic acid	—	24
4		Unknown	Miscellaneous unsequenced enzymes that do not fit into other groups	—	9

Table adapted from Bush (2001); ^aSee <http://www.lahey.org/Studies> for current list of TEM, SHV and OXA beta-lactamases (216 variants in 2005).

clavulanic acid placed 20 mm from disks containing one or more oxyimino-aminothiazole-beta-lactam antimicrobial drugs (Tzelepi et al., 2000).

The issues revealed by susceptibility testing have led to some confusion about the clinical meaning of an MIC in the susceptible range for a bacterium producing an ESBL (Rupp and Fey, 2003). It has been known for years that the high concentrations of antimicrobials achieved in urine mean that usually accepted "breakpoint" criteria for bacterial susceptibility cannot be applied to urinary tract infections, and ESBL-producing bacteria are often no exception (Emery and Weymouth, 1997). However, a review of clinical outcome in human patients infected with an ESBL-producing bacteria in which susceptibility testing indicated full susceptibility, not resistance, showed cephalosporin treatment failure in over half (Paterson et al., 2001). It is thus currently recommended that any bacterium producing an ESBL be regarded as resistant to all extended-spectrum beta-lactam antibiotics.

Beta-lactamase Inhibitors

The inhibitors (clavulanic acid, sulbactam, tazobactam) have high substrate specificity for a wide variety

of beta-lactamases. Their binding to these inhibitors is irreversible, thus allowing the active beta-lactam (amoxicillin, piperacillin, etc) to kill the organism since beta-lactamase is effectively absent. The spectrum of activity of these inhibitors is described in Table 9.1; clavulanic acid and tazobactam have a similar spectrum. The efficacy of these inhibitors is shown in Table 9.2.

Bibliography

- Amyes SGB, Miles RS. 1998. Extended-spectrum β -lactamases: the role of inhibitors in therapy. *J Antimicrob Chemother* 42:415.
- Bradford PA. 2001. Extended-spectrum β -lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev* 14:933.
- Bush K. 2001. New β -lactamases in Gram-negative bacteria: Diversity and impact on the selection of antimicrobial therapy. *Clin Infect Dis* 32:1085.
- Emery CL, Weymouth LA. 1997. Detection and clinical significance of extended-spectrum β -lactamases in a tertiary-care medical center. *J Clin Microbiol* 35:2061.
- Girard AE, et al. 1987. Activity of beta-lactamase inhibitor sulbactam plus ampicillin against animal isolates of

Table 9.2. Activity (MIC₉₀, µg/ml) of amoxicillin and ampicillin with or without clavulanic acid and sulbactam, respectively.

Organism	Amoxicillin	Amoxicillin-clavulanic acid	Ampicillin	Ampicillin-sulbactam
Gram-positive aerobes				
<i>Staphylococcus aureus</i>	64	8	16	1
Gram-negative aerobes				
<i>Enterobacter</i> spp.	> 128	> 128	> 128	> 128
<i>Escherichia coli</i>	> 128	8	> 128	8
<i>Klebsiella</i> spp.	> 128	1	> 128	8
<i>Proteus</i> spp.	> 128	> 128	> 128	8
<i>Pseudomonas aeruginosa</i>	> 128	> 128	> 128	> 128
<i>Salmonella</i> spp.	> 16	< 1		
Gram-positive anaerobes				
<i>Clostridium</i> spp.	> 256	16	0.3	16
Gram-negative anaerobes				
<i>Bacteroides fragilis</i>	256	4	32	0.5
<i>Bacteroides</i> spp.	32	1	0.5	1
<i>Fusobacterium</i> spp.	16	4	16	8

Source: C. Hoare, Smith Kline Beecham (unpublished observations, with permission and additions).

Pasteurella, *Haemophilus*, *Staphylococcus*. Am J Vet Res 48:1677.

Livermore DM. 1995. β -lactamases in laboratory and clinical resistance. Clin Microbiol Rev 8:557.

National Council on Clinical Laboratory Standards. 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A5 and informational supplement M100-S10. National Council for Clinical Laboratory Standards, Wayne, PA.

Paterson DL, et al. 2001. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum beta-lactamases: Implications for the clinical microbiology laboratory. J Clin Microbiol 39:2206.

Petrosino J, et al. 1998. β -lactamases: protein evolution in real time. Trends Microbiol 6:323.

Rice LB, et al. 1991. Efficacy of different β -lactams against an extended-spectrum β -lactamase-producing *Klebsiella pneumoniae* strain in the rat intra-abdominal abscess model. Antimicrob Agents Chemother 35:1243.

Rupp ME, Fey PD. 2003 Extended-spectrum β -lactamases (ESBL)-producing Enterobacteriaceae. Drugs 63:353.

Tzelepi E, et al. 2000. Isolation of an SHV-12 beta-lactamase-producing *Escherichia coli* from a dog with recurrent urinary tract infections. Antimicrob Agents Chemother 44:3483.

Clavulanic Acid

Clavulanic acid is a synthetic compound with a bicyclic nucleus similar to that of a penicillin, except for an oxygen in place of the sulfur and a missing acylamino side chain at position 6. It has good affinity for the majority of plasmid-mediated beta-lactamases

(Table 9.1) and all chromosomally mediated penicillinases, but little affinity for chromosomal cephalosporinases. This latter group of enzymes usually hydrolyzes amoxicillin and ticarcillin, with which clavulanic acid is combined, poorly. Clavulanic acid is combined with amoxicillin in the ratio of 4:1 in veterinary formulations. The amoxicillin:clavulanic acid ratios vary between products and sizes of human formulations (suspension, tablets and chewable tablets), so they are not interchangeable with veterinary formulations. Ticarcillin is now available in a human injectable formulation with a ticarcillin:clavulanic acid ratio of 15:1. The combinations are usually bactericidal at one or two dilutions below the MIC of amoxicillin or ticarcillin used alone.

Clavulanic Acid-Amoxicillin

Antibacterial Activity. Amoxicillin-clavulanic acid has a spectrum of activity similar to that of a first or second generation cephalosporin.

Good susceptibility (MIC \geq 8/4 µg/ml, *S. aureus* \geq 4/2 µg/ml [first figure refers to amoxicillin component of the combination]) is shown with several bacteria: excellent susceptibility of Gram-positive bacteria, including beta-lactamase-producing *S. aureus*. Fastidious Gram-negative bacteria (*Actinobacillus* spp., *Bordetella* spp., *Haemophilus* spp., *Pasteurella* spp.) are susceptible, including strains resistant to amoxicillin.

Table 9.3. Activity of amoxicillin-clavulanic acid (MIC₉₀, µg/ml) against selected veterinary pathogens.

Organism	MIC ₉₀	Organism	MIC ₉₀
Gram-positive cocci			
<i>Staphylococcus aureus</i>	0.5	<i>Streptococcus dysgalactiae</i>	≤0.13
<i>Staphylococcus intermedius</i>	0.25	<i>Streptococcus suis</i>	≤0.13
<i>Streptococcus agalactiae</i>	≤0.13		
Gram-positive rods			
<i>Arcanobacterium pyogenes</i>	0.25	<i>Listeria monocytogenes</i>	0.25
Gram-negative aerobes			
<i>Actinobacillus pleuropneumoniae</i>	0.5	<i>Pasteurella multocida</i>	0.25
<i>Bordetella bronchiseptica</i>	2	<i>Pseudomonas</i> spp.	≥32
<i>Escherichia coli</i>	8	<i>Proteus mirabilis</i>	0.5
<i>Histophilus somni</i>	0.06	<i>Salmonella</i> spp.	2
<i>Moraxella bovis</i>	0.06		
<i>Mannheimia haemolytica</i>	0.13		
Anaerobic bacteria			
<i>Bacteroides fragilis</i>	0.5	<i>Porphyromonas asaccharolytica</i>	1.0
<i>Clostridium perfringens</i>	0.5	<i>Fusobacterium</i> spp.	≥32

Source: C. Hoare, Smith Kline Beecham (unpublished observations, with permission and additions).

Enterobacteriaceae such as *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., and *Salmonella* spp. are usually susceptible; most anaerobes, including *Bacteroides fragilis*, are susceptible (Table 9.3).

Variable susceptibility is found in some *E. coli* and *Klebsiella* spp.

Resistance (MIC ≥ 32/16 µg/ml [first figure refers to amoxicillin component]) is shown among *Citrobacter* spp., *Enterobacter* spp., *P. aeruginosa*, *Serratia* spp., and methicillin-resistant *S. aureus*.

Antibiotic Resistance. Clavulanic acid may induce beta-lactamases in susceptible *Providencia* and *Enterobacter*. Until recently, emergence of resistance to clavulanic acid had not been a problem in bacteria isolated from animals. However, a variety of resistance mechanisms have emerged (Table 9.1). These include plasmid-encoded functional group 1 CMY, FOX and other families of beta-lactamases that do not bind to clavulanic acid, hyperproduction of TEM beta-lactamases, reduced antibiotic uptake, and production of inhibitor-resistant (IRT) beta-lactamases derived from TEM-1.

Pharmacokinetic Properties. Clavulanic acid is well-absorbed after oral administration and has pharmacokinetic properties similar to amoxicillin. Tissue distribution in extracellular fluids is widespread but

penetration into milk and into cerebrospinal fluid is relatively poor, unless inflammation is present. The elimination half-life is about 75 minutes. The drug is largely eliminated unchanged in the urine, with some biliary excretion. In dogs and cats, higher than recommended doses appear to show an inhibitory effect of amoxicillin on the absorption of the clavulanate component (Vree et al., 2002, 2003), but the significance of this observation is unclear.

Toxicity and Side Effects. The combination is well tolerated. The major side effects to oral administration, reported in about 10% of human patients, are nausea, vomiting, and diarrhea. These are due to a direct effect of clavulanic acid on gastrointestinal motility, so recommended oral doses should not be exceeded. Mild gastrointestinal upset has been reported in dogs and cats. Other side effects are typical for penicillins in general. The combination should not be used in penicillin- or cephalosporin-sensitive animals. It should not be administered orally to herbivores or by injection to horses. It must also not be used in rabbits, guinea pigs, hamsters, or gerbils.

Administration and Dosage. Recommended dosage is shown in Table 9.4. The manufacturers' recommendations for once-daily dosing of parenteral products in food animals is underdosing. Twice-daily or more frequent administration takes advantage of the time-

Table 9.4. Suggested dosage of clavulanic acid, sulbactam or tazobactam potentiated penicillins.

Drug	Species	Route	Dose (mg/kg)	Interval
Clavulanate-amoxicillin	Dogs, cats	PO	12.5-20	8-12
		SC	10	8
	Cattle	IM	7	12-24
	Preruminant calves	PO	5-10	12
	Sheep	IM	8.75	12-24
Clavulanate-ticarcillin	Dogs, cats	IV	40-50	6-8
	Horses	IV	50	6
Sulbactam-ampicillin	Cattle	IM	10	24
Piperacillin-tazobactam	Dogs, cats	IV	4	6

dependent efficacy of beta-lactam drugs. Clavulanic acid is highly sensitive to moisture, so precautions must be taken to ensure dryness during storage.

Clinical Applications. Clavulanic acid-amoxicillin is a valuable addition as an orally administered antibiotic in monogastrates. It extends the range of amoxicillin against beta-lactamase-producing common opportunist pathogens, including fastidious organisms, Enterobacteriaceae, and aerobic bacteria. It is not effective against *P. aeruginosa*. Some *E. coli*, *Proteus*, and *Klebsiella* spp. are only susceptible to urinary concentrations of the combination, so that it is recommended for empirical treatment of urinary tract infections in dogs and cats. Activity against anaerobes is a particularly useful attribute.

Cattle, Sheep, and Goats. Clavulanic acid-amoxicillin has been introduced in Europe as an IM injectable with amoxicillin trihydrate for cattle and an oral bolus for calves. Uses include the treatment of lower respiratory tract infections, anaerobic soft tissue infections, and neonatal calf diarrhea caused by *E. coli* and *Salmonella*. The dosage recommended for oral treatment of *E. coli* diarrhea in calves was 12.5 mg combined drug/kg every 12 hours for at least 3 days (Constable, 2004). Dual parenteral and intramammary administration to treat clinical mastitis in cows gave improved results over intramammary use alone (Perner et al., 2002). In sheep, the combination can be recommended in the treatment of pasteurellosis (Gilmour et al., 1990). There are few published data on pharmacokinetic behavior of the drug in ruminants, but the dosing rate recommended by the manufacturer (Table 9.4) appears low. There may therefore be advantage to at least twice daily injection of the rec-

ommended dose. Plasmid (CMY2 beta-lactamase) mediated resistance to the combination has been described in multidrug-resistant *Salmonella* of several serovars derived from cattle, including Newport (Zhao et al., 2001, 2003). Several large plasmids appear to be responsible for the dissemination of the CMY2 gene in *Salmonella* (Giles et al., 2004).

Swine. The combination has potential application in the treatment of a variety of infections in swine caused by plasmid-mediated beta-lactamase-producing bacteria, possibly including neonatal diarrheal *E. coli* (Webster, 1990). A broader range of porcine clinical trials of this drug are needed.

Dogs and Cats. Clavulanic acid-amoxicillin has many applications in dogs and cats, with the advantage of twice-daily oral administration for medication by owners. Among other applications are skin and soft tissue infections caused by *S. aureus* and *S. intermedius* and infections following bite wounds that involve mixed bacteria including anaerobes, upper and lower respiratory tract infections, anal sacculitis, gingivitis, and urinary tract infections involving common opportunist bacteria (*S. aureus*, *E. coli*, *Proteus*, *Klebsiella*). Apart from urinary tract infections, the drug is not recommended for serious infections caused by *S. aureus*, *E. coli*, *Proteus*, or *Klebsiella*, since tissue concentrations may not exceed some strains' MIC for a sufficient part of the dosing interval. Interestingly, however, doubling the dose was not associated with increased cure rates in the treatment of canine pyoderma (Lloyd et al., 1997). The combination was not as effective as clindamycin for treatment of superficial pyoderma (Littlewood et al., 1999). First-generation cephalosporins have proven efficacy and, with a nar-

rower spectrum, are less likely to select for resistance in *S. aureus* and other pathogens. Clavulanic acid-amoxicillin was less effective than the fluoroquinolone marbofloxacin in treating urinary tract infections in dogs (Cotard et al., 1995). For treatment of *Bordetella* infections, the combination is preferred to amoxicillin alone because isolates are less likely to be resistant to the combination (Speakman et al., 2000). Clavulanic acid-amoxicillin may have particular value in peritonitis associated with intestinal content spillage because of the combination's activity against enteric bacteria, including anaerobes.

In spite of the poor ability of beta-lactams to penetrate membranes, the combination showed unexpected efficacy in treating *Chlamydia psittaci* infection in cats, which exceeded that of doxycycline (Sturgess et al., 2001). However, unlike doxycycline treated cats, infection recurred in some cats treated with clavulanic acid-amoxicillin. Treatment for four weeks is therefore recommended.

Bibliography

- Constable PD. 2004. Antimicrobial use in the treatment of calf diarrhea. *J Vet Intern Med* 18:8.
- Cotard JP, et al. 1995. Comparative study of marbofloxacin and amoxicillin-clavulanic acid in the treatment of urinary tract infections in dogs. *J Small Anim Pract* 36:349.
- Giles WP, et al. 2004. DNA sequence of regions surrounding bla_{CMY-2} from multiple *Salmonella* plasmid backbones. *Antimicrob Agents Chemother* 48:2845.
- Jones RL, et al. 1994. Clinical observations on the use of oral amoxycillin/clavulanate in the treatment of gingivitis in dogs and cats and anal sacculitis in dogs. *Br Vet J* 150:385.
- Littlewood JD, et al. Clindamycin hydrochloride and clavulanate-amoxycillin in the treatment of canine superficial pyoderma. *Vet Rec* 144:662.
- Lloyd DH, et al. 1997. Treatment of canine pyoderma with co-amoxyclov: a comparison of two dose rates. *Vet Rec* 141:439.
- Perner J, et al. 2002. Retrospective study using Synulox® in mastitis therapy. *Tierärztliche Praxis* 30:286.
- Speakman AJ, et al. Antibiotic susceptibility of canine *Bordetella bronchiseptica* isolates. *Vet Microbiol* 71:193.
- Sturgess CP, et al. 2001. Controlled study of the efficacy of clavulanic acid-potentiated amoxycillin in the treatment of *Chlamydia psittaci* in cats. *Vet Rec* 149:73.
- Vree TB, et al. 2002. Variable absorption of clavulanic acid after an oral dose of 25 mg/kg of Clavubactin and Synulox in healthy cats. *Scientific World Journal* 2:1369.
- Vree TB, et al. 2003. Variable absorption of clavulanic acid after an oral dose of 25 mg/kg of Clavubactin® and Synulox® in healthy dogs. *J Vet Pharmacol Ther* 26:165.
- Webster CJ. 1990. Parenteral amoxycillin-clavulanate in the treatment of diarrhea in young pigs. *Vet Rec* 126:263.
- Winokur PL, et al. Evidence of transfer of CMY-2 AmpC β -lactamase plasmids between *Escherichia coli* and *Salmonella* isolates from food animals and humans. *Antimicrob Agents Chemother* 45:2716.
- Zhao S, et al. 2001. Identification and expression of cephamycinase bla_{CMY} genes in *Escherichia coli* and *Salmonella* isolates from food animals and ground meat. *Antimicrob Agents Chemother* 45:3647.
- Zhao S, et al. 2003. Characterization of *Salmonella enterica* serotype Newport isolated from humans and food animals. *J Clin Microbiol* 41:5366.

Clavulanic Acid-Ticarcillin

Clavulanic acid-ticarcillin is available as a parenteral (usually IV) drug for use in human medicine. It offers the advantage over clavulanic acid-amoxicillin of the greater activity of ticarcillin against *Enterobacter* and *P. aeruginosa*. The combination has good activity against the majority of ticarcillin-resistant Enterobacteriaceae, *S. aureus*, anaerobes including *B. fragilis*, and many *P. aeruginosa*. However, the MIC₉₀ of bacterial isolates from disease processes, especially *Enterobacter*, *E. coli*, and *Klebsiella*, is on the high end of the susceptibility range (MIC \leq 16 μ g/ml) or in the moderately susceptible range (MIC 32–64 μ g/ml) (Sparks et al., 1988). No potentiating activity occurs with the combination for *Enterobacter*, *P. aeruginosa*, and *Serratia*, and results of treatment of human clinical infections caused by these organisms have sometimes been disappointing, possibly because of induction of beta-lactamases by the clavulanate component. The combination has the disadvantage in animals of requiring frequent (every 8 hour) IV dosage (Table 9.4), although a 12-hour dosing interval may be used in neonatal foals. In human medicine, it is used in the empirical treatment of serious infections in immunocompromised patients when combined with an aminoglycoside. Because of need for IV dosage, applications of clavulanic acid-ticarcillin in veterinary medicine are few.

Bibliography

- Garg RC, et al. 1987. Serum levels and pharmacokinetics of ticarcillin and clavulanic acid in dogs following parenteral administration of timentin. *J Vet Pharmacol Ther* 10:324.
- Sanders C, Cavalieri SJ. 1990. Relevant breakpoints for ticarcillin-clavulanic acid should be set primarily with data from ticarcillin-resistant strains. *J Clin Microbiol* 28:830.

Sparks SE, et al. 1988. In vitro susceptibility of bacteria to a ticarcillin-clavulanic acid combination. *Am J Vet Res* 49:2038.

Sweeney RW, et al. 1988. Pharmacokinetics of intravenously and intramuscularly administered ticarcillin and clavulanic acid in foals. *Am J Vet Res* 49:23.

Wilson WD, et al. 1991. Pharmacokinetics and bioavailability of ticarcillin and clavulanate in foals after intravenous and intramuscular administration. *J Vet Pharmacol Ther* 14:78.

Sulbactam

Sulbactam (penicillanic acid sulfone) is a synthetic derivative of 6-aminopenicillanic acid. It is poorly absorbed orally, but a double ester linkage of sulbactam with ampicillin has been developed to produce the prodrug sultamicillin, which is well-absorbed orally and releases the two drugs in the intestinal wall. Sulbactam has no antibacterial activity by itself but irreversibly binds the same groups of beta-lactamases as clavulanic acid, though sulbactam's affinity is several times lower. It also binds beta-lactamases of *Citrobacter*, *Enterobacter*, *Proteus*, and *Serratia* that clavulanic acid does not. The same level of inhibition as clavulanic acid can be achieved by increasing the concentration of sulbactam (2:1) for clinical use. Sulbactam is combined with ampicillin in part because of pharmacokinetic similarities, but has also been combined with cefoperazone.

Sulbactam-Ampicillin

Antibacterial activity is slightly broader but is marginally lower than that of clavulanic acid-amoxicillin (Tables 9.1, 9.2). Sulbactam-ampicillin's lower affinity for beta-lactamases may limit its activity against some potent beta-lactamase-producing bacteria (Gisby and Beale, 1988).

Pharmacokinetic properties are similar to those of amoxicillin-clavulanic acid, but sulbactam is poorly absorbed orally. It is available for use in human medicine as the orally absorbed prodrug sultamicillin. The combination is well-absorbed after IM injection, distributes well into tissues in the extracellular space, and penetrates CSF through inflamed meninges. Penetration into milk is modest. Elimination is largely in the urine. The half-life is about 1 hour. Pharmacokinetic studies in calves (Fernández-Varón et al., 2005) and in sheep (Escudero et al., 1999) have suggested that the ampicillin concentration should be increased since sulbactam is more slowly eliminated than ampicillin.

The combination used for parenteral injection is well-tolerated and the side effects are those of penicillins generally, without the diarrhea that may occur with the orally administered clavulanic acid-amoxicillin. Intramuscular injection may be painful. The combination should not be used in herbivores with expanded large intestines (horses, rabbits, hamsters, guinea pigs), although adverse effects were not observed in foals (Hoffman et al., 1992).

Clinical applications. Sulbactam-ampicillin restores and extends the antibacterial activity of ampicillin to include common bacteria that have acquired beta-lactamases. Sulbactam-ampicillin is no longer available for veterinary use in North America, but is available in some other countries, where it is used for the treatment of bovine respiratory disease for its activity against *Pasteurella* (including beta-lactamase-producing strains), *Histophilus somni*, *Arcanobacterium pyogenes*, and opportunist bacteria, including *E. coli*. The efficacy and superiority of the combination to ampicillin alone has been demonstrated in experimental and field studies (Bentley and Cummins, 1987; Grimshaw et al., 1987a; Gifford et al., 1988). It was as efficacious as ceftiofur in the treatment of bovine respiratory disease in one study (Schumann and Janzen, 1991). Its advantage over ampicillin in the parenteral treatment of undifferentiated diarrhea in neonatal calves has been demonstrated (Grimshaw et al., 1987b). Once-daily dosage in cattle, while clinically effective, is underdosing based on pharmacokinetic and pharmacodynamic considerations. This combination may also see extralabel use for diseases such as salmonellosis; however, there have been no clinical trials reporting its use for diseases other than undifferentiated bovine respiratory disease and enteric colibacillosis. Others of the many potential clinical applications are described for cattle under benzyl penicillin and clavulanic acid-amoxicillin, with the combination clearly having the advantage over benzyl penicillin for many applications. Suggested dosing is shown in Table 9.4.

Bibliography

Bentley OE, Cummins JM. 1987. Efficacy of sulbactam, a beta-lactamase inhibitor, combined with ampicillin in the therapy of ampicillin-resistant pneumonic pasteurellosis in feedlot calves. *Can Vet J* 28:591.

- Escudero E, et al. 1999. Pharmacokinetics of an ampicillin-sulbactam combination after intravenous and intramuscular administration to sheep. *Can J Vet Res* 63:25.
- Fernández-Varón E, et al. 2005. Pharmacokinetics of an ampicillin-sulbactam combination after intravenous and intramuscular administration to neonatal calves. *Vet J* 169:437.
- Gifford GA, et al. 1988. Clinical and pathological evaluation of sulbactam/ampicillin for treatment of experimental bovine pneumonic pasteurellosis. *Can Vet J* 29:142.
- Grimshaw WTR, et al. 1987a. The efficacy of sulbactam-ampicillin in the therapy of respiratory disease associated with ampicillin resistant *Pasteurella* species in housed cattle. *Vet Rec* 121:393.
- Grimshaw WTR, et al. 1987b. Efficacy of sulbactam-ampicillin in the treatment of neonatal calf diarrhea. *Vet Rec* 121:162.
- Hoffman AM, et al. 1991. Evaluation of sulbactam plus ampicillin for treatment of experimentally induced *Klebsiella pneumoniae* lung infection in foals. *Am J Vet Res* 53:1059.
- Schumann FJ, Janzen ED. 1991. Comparison of ceftiofur sodium and sulbactam-ampicillin in the treatment of bovine respiratory disease. *Wien Tierarzt Wschr* 78:185.

Piperacillin-Tazobactam

Tazobactam is a beta-lactamase inhibitor with activity similar to but broader than clavulanic acid and sulbactam. For example, it resists hydrolysis by Bush group 1 and group 3 beta-lactamases, as well as beta-lactamases inhibited by clavulanic acid (Table 9.1). Unlike clavulanic acid, it is only a poor to moderate inducer of beta-lactamases. Combined with piperacillin in an 8:1 ratio (piperacillin:tazobactam), it has considerably enhanced the activity of this group 5 (antipseudomonal) penicillin against beta-lactamase producing bacteria.

Piperacillin-tazobactam possesses broad-spectrum activity against many Enterobacteriaceae and other Gram-negative bacteria. Minor exceptions include *Enterobacter* spp. and *Xanthomonas maltophilia*. Activity against anaerobic bacteria such as *B. fragilis*, including cefoxitin-resistant *B. fragilis*, is an important feature of the combination. It is active against a wide range of Gram-positive bacteria. Pharmacokinetic properties are typical of beta-lactam drugs generally.

Indications in human medicine are generally those of third-generation cephalosporins, with an emphasis on the additional beneficial effects of this combination against anaerobic bacteria. It rivals imipenem in breadth of antibacterial activity. The combination is used in treatment of intra-abdominal infections (where mixed aerobic-anaerobic infections are likely

to be present) and other polymicrobial infections. It is as effective for this purpose as imipenem or clindamycin-gentamicin. Piperacillin-tazobactam is also used in the treatment of fever in neutropenic patients (in combination with an aminoglycoside). Its advantage over a ticarcillin-clavulanate combination in the treatment of human community-acquired lower respiratory infection has been convincingly demonstrated. While there are few indications for use in animals of this broad-spectrum drug, empirical dosage is suggested in Table 9.4.

Bibliography

- Bryson HM, Brogden RN. 1994. Piperacillin/tazobactam. *Drugs* 47:506.
- Sanders EW, Sanders CC. 1996. Piperacillin/tazobactam: a critical review of the evolving clinical literature. *Clin Infect Dis* 22:107.

Carbapenems: Imipenem-Cilastatin, Meropenem, and Biapenem

Carbapenems (Figure 7.2) are derivatives of *Streptomyces* spp. that differ from penam penicillins by the substitution of a CH₂ group for the sulphur in the five-membered ring attached to the beta-lactam ring. They currently have the widest activity of any antibiotics, being highly active against a wide variety of Gram-positive and Gram-negative bacteria and resistant to many beta-lactamases. N-formimidoyl thienamycin (imipenem) is stable to bacterial beta-lactamases other than the Bush group 3 beta-lactamases. Its hydrolysis by a dihydropeptidase in the kidney is overcome by 1:1 combination with cilastatin, a dihydropeptidase inhibitor. Other semisynthetic carbapenems, meropenem and biapenem, have activity similar to imipenem but resist degradation by renal dihydropeptidase.

Antibacterial Activity

Carbapenems are active against almost all clinically important aerobic or anaerobic Gram-positive or Gram-negative cocci or rods. Individual species may be resistant. They offer the advantages of broad antimicrobial activity and, by comparison to third and fourth generation cephalosporins, resistance to Bush groups 1 and 2 beta-lactamases. Biapenem and

meropenem are slightly less active than imipenem against Gram-positive bacteria but equivalent or slightly more active against Gram-negative aerobes.

Good susceptibility ($MIC \leq 4 \mu\text{g/ml}$) is shown by most pathogenic bacteria, which includes most Gram-positive bacteria; imipenem is highly active against Gram-positive cocci (including most enterococci), similar to that of benzyl penicillin. *Mycobacterium avium-intracellulare*, *Nocardia* spp., and *Brucella* spp. are susceptible. Carbapenems are highly active against anaerobic bacteria, including *B. fragilis*. These drugs are the most active of the beta-lactam antibiotics against Gram-negative bacteria. Their activity includes beta-lactamase-producing fastidious organisms, Enterobacteriaceae including beta-lactamase-producing isolates, and most *P. aeruginosa*. They are slightly less active against *Proteus* spp. than against other enteric organisms.

Resistance ($MIC \geq 16 \mu\text{g/ml}$) is shown by methicillin-resistant *S. aureus*, *Burkholderia cepacia*, and by some Enterobacter spp., Aeromonas spp., *P. aeruginosa*, *P. maltophilia*, and Enterococcus faecium.

Antibiotic Resistance

Resistance during therapy with imipenem has been commonly reported in *P. aeruginosa* and attributed to alterations in outer-membrane proteins, which reduce permeability. Many of these isolates are susceptible to meropenem. Resistance through metalloenzyme carbapenemases occurs in some isolates from the species listed under Resistance above. Stably derepressed high-level beta-lactamase producing mutants of *Citrobacter* spp., Enterobacter spp., and Serratia spp. may be more susceptible to carbapenems than to third- and fourth-generation cephalosporins.

Pharmacokinetic Properties

The carbapenems are not absorbed after oral administration, although orally administered carbapenems are being developed. Following IV administration, they are widely distributed to extracellular fluid throughout the body and reach therapeutic concentrations in most tissues in humans. There is poor penetration into cerebrospinal fluid, even with inflamed meninges. Carbapenems have the low volume of distribution typical of beta-lactam drugs. Imipenem is almost exclusively eliminated through the kidneys, being me-

tabolized in renal tubules by a dihydropeptidase enzyme. Addition of cilastatin prevents this metabolism. This increases the elimination half-life and allows the drug to be excreted in large amounts in active form into urine. Meropenem, by contrast, is stable to dihydropeptidase. The half-life of these carbapenems is about 1 hour.

Toxicity and Side Effects

The most common side effects in human patients have been gastrointestinal disturbance (nausea, vomiting, diarrhea) in about 4% of patients, hypersensitivity reactions (rash) in about 3% of patients, and, for imipenem, seizures in about 0.5% of patients. (These last are associated with high doses, renal failure, or underlying neurological abnormalities.) Hypersalivation was noted in dogs given rapid IV infusion. Vocalization, presumably indicating pain, was noted in 1 and 2 of 6 dogs administered the drug IM or SC, respectively (Barker et al., 2003). Liver enzymes may rise transiently during treatment. Meropenem use in people is associated with a lower incidence of gastrointestinal disturbance than imipenem, and does not cause seizures.

Drug Interactions

Carbapenems may be synergistic with aminoglycosides against *P. aeruginosa*. Rapid emergence of resistance in *P. aeruginosa* (about 20%) during treatment with imipenem suggests that it should be combined with an aminoglycoside for infections with this organism, although the combination may not prevent the emergence of resistance.

Administration and Dosage

Imipenem is administered IV (over 20–30 minutes) or by deep IM injection, every 8 hours. Dosage in dogs and cats, for which it is used occasionally, is largely empirical, in the range 5–10 mg/kg every 8 hours. The drug may be given SC as well as IM in dogs (Barker et al., 2003), although this may be painful. In horses, Orsini et al. (2005) recommended a higher IV dosage of 10–20 mg/kg every 6 hours for treatment of susceptible infections.

Meropenem is usually administered IV; an empiric dosage is 5–10 mg/kg every 8 hours. Bidgood and Papich (2002) did not observe painful effects of SC administration in dogs and suggested a dosage of 8–12 mg/kg SC every 8 or every 12 hours depending on the susceptibility of the organism being treated.

Clinical Applications

These extraordinary antimicrobial drugs are used in human medicine in the treatment of hospital-acquired infections caused by multiresistant Gram-negative bacteria, or mixed aerobic and anaerobic infections. They are used successfully in human patients for intra-abdominal infections (less effective than piperacillin-tazobactam but equivalent to clindamycin-tobramycin or cefotaxime-metronidazole), severe lower respiratory tract infections (as or more effective than third-generation cephalosporin-amikacin treatment), septicemia (equivalent to ceftazidime-amikacin in febrile neutropenic patients), life-threatening soft tissue infections, and osteomyelitis. Imipenem is not recommended for the treatment of bacterial meningitis or *P. aeruginosa* infection. Meropenem is as effective as cefotaxime or ceftriaxone in treatment of bacterial meningitis in people.

Carbapenems should be reserved for the treatment of infections caused by cephalosporin-resistant Enterobacteriaceae and for empirical treatment of febrile illness in neutropenic patients (Chapter 23). Their use should be limited in veterinary medicine. The potential for emergence of *P. aeruginosa* resistant to imipenem suggests that administration of imipenem with an aminoglycoside would be prudent. The growing tendency of small animal intensive care units to use imipenem as a first line antibacterial drug in seriously ill animals with undiagnosed infection is likely to result in progressive development of resistant nosocomial infections in these settings.

Bibliography

- Balfour JA, et al. 1996. Imipenem/cilastatin. *Drugs* 51:99.
 Barker CW, et al. Pharmacokinetics of imipenem in dogs. *Am J Vet Res* 64:694.
 Bidgood T, Papich MG. 2002. Plasma pharmacokinetics and tissue fluid concentrations of meropenem after intravenous and subcutaneous administration in dogs. *Am J Vet Res* 63:1622.
 Jaccard C, et al. 1998. Prospective randomized comparison of imipenem-cilastatin and piperacillin-tazobactam in nosocomial pneumonia or peritonitis. *Antimicrob Agents Chemother* 42:2966.
 Orsini JA, Perkins S. 1994. New beta-lactam antibiotics in critical care medicine. *Comp Cont Ed Pract Vet* 16:183.
 Orsini JA, et al. 2005. Pharmacokinetics of imipenem-cilastatin following intravenous administration in health adult horses. *J Vet Pharmacol Ther* 28:355.

- Rasmussen BA, Bush K. 1997. Carbapenem-hydrolyzing β -lactamases. *Antimicrob Agents Chemother* 41:223.
 Wiseman LR, et al. 1995. Meropenem. *Drugs* 50:73.

Monobactams: Aztreonam

Monobactams possess the simple beta-lactam ring without the attached thiazolidine ring (Figure 7.2). Aztreonam was the first monobactam introduced into human medicine. Other monobactams such as tigemonam, which can be administered orally, are in clinical trials in human medicine. Aztreonam is a synthetic analogue of an antibiotic isolated from a *Streptomyces* species. It binds mainly to PBP3, disrupting cell-wall synthesis, and is stable to most beta-lactamases. Comments below are largely confined to aztreonam.

Antibacterial Activity

Good susceptibility ($MIC \leq 8 \mu\text{g/ml}$) is limited by PBP3 binding to almost all Gram-negative aerobic bacteria, particularly fastidious organisms (*Haemophilus* spp., *Pasteurella* spp.) and Enterobacteriaceae. The susceptibility of *P. aeruginosa* is variable.

Resistance ($MIC \geq 32 \mu\text{g/ml}$) is shown in Gram-positive bacteria and anaerobic bacteria; other *Pseudomonas* spp., *B. cepacia*, *Citrobacter* spp., and *Enterobacter* spp. are often resistant because they are susceptible to extended-spectrum (Bush group 2be) beta-lactamases.

Antibiotic Resistance

Aztreonam is hydrolysed by extended-spectrum beta-lactamase producers but is resistant to Bush group 1 cephalosporinases.

Pharmacokinetic Properties

Aztreonam is not absorbed after oral administration. It is rapidly absorbed after IM injection in human patients and distributes widely in extracellular fluid throughout the body. Penetration into the cerebrospinal fluid of human patients with meningitis has achieved concentrations that should eliminate infections with Enterobacteriaceae. The elimination half-life is about 1.6 hours in people; elimination is mainly renal.

Toxicity and Side Effects

Toxicity is similar to that of benzyl penicillin, with no apparent cross-allergy in human patients allergic to penicillins or cephalosporins. These drugs do not cause the gastrointestinal disturbances associated with carbapenems and other broad-spectrum beta-lactam antibiotics. Their inactivity against Gram-positive bacteria may lead to superinfection with yeasts and with Gram-positive aerobes, including *Enterococcus* spp. and *S. aureus*.

Drug Interactions

Aztreonam is often synergistic with aminoglycosides, including aminoglycoside-resistant Gram-negative bacteria and *P. aeruginosa*. This may have little advantage since aztreonam is often used clinically as a substitute for an aminoglycoside. Aztreonam may have advantage combined with beta-lactams susceptible to Bush group 1 cephalosporinases, since it is poorly inactivated by these enzymes.

Administration and Dosage

Aztreonam is administered IV (over 3–5 minutes) or IM. An empirical dose in animals is 30–50 mg/kg every 8 hours.

Clinical Applications

The narrow spectrum of aztreonam precludes its use in human medicine for empirical treatment of infections, except possibly for urinary tract infections. It has the potential to substitute for the more toxic aminoglycosides in combination therapy, for example

with clindamycin or metronidazole in serious, mixed anaerobic infections or with erythromycin in mixed infections where Gram-positive bacteria may be present. Aztreonam is used on its own in a wide variety of infections involving Gram-negative bacteria (urinary tract, lower respiratory tract, septicemia) with success as a relatively nontoxic drug in human medicine, including in seriously ill, immunocompromised patients infected with multiresistant Gram-negative aerobes. Its place in veterinary medicine appears to be slight but might include treatment of meningitis in neonatal animals.

Bibliography

- Chin N-X, Neu HC. 1988. Tigemonam, an oral monobactam. *Antimicrob Agents Chemother* 32:84.
 Lister PD, et al. 1998. Cefepime-aztreonam: a unique double β -lactam combination for *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 42:1610.
 Neu HC (ed). 1990. Aztreonam's role in the treatment of gram-negative infection. *Am J Med* 88(Suppl):3C.
 Rubinstein E, Isturiz R. 1991. Aztreonam: The expanding clinical profile. *Rev Infect Dis* 13:S581.

Tribactams

Tribactams have a tricyclic structure related to that of carbapenems. Sanfetrinem cilexetil is the prodrug of sanfetrinem and is administered orally in people. It has high stability to many beta-lactamases and a broad-spectrum of activity similar to that of carbapenems.

Peptide Antibiotics: Polymyxins, Glycopeptides, and Bacitracin

Patricia M. Dowling

Polymyxins, glycopeptides, and bacitracin are peptide antibiotics with a variety of actions against bacteria. Streptogramins are also peptides but are discussed in Chapter 11 because of their common mechanism of action with lincosamides. Because they are systemically toxic, the clinical development of polymyxins and bacitracin has not been pursued since their discovery early in the antibiotic era. However, because of the worldwide increase in multidrug-resistant Gram-negative organisms, the polymyxins are being re-evaluated for clinical use (Li et al., 2005). Glycopeptides and streptogramins are of great interest, particularly in human medicine, because of their activity against Gram-positive bacteria, including multidrug-resistant enterococci.

Polymyxins

Polymyxins are antibiotic products of *Bacillus polymyxa*. When first described in the 1940s they were of great interest for their activity against *Pseudomonas aeruginosa*. They are most commonly used topically because of their systemic toxicity. There is interest in their systemic use at subantimicrobial doses for binding and inactivating endotoxin, especially in horses.

Chemistry

Polymyxins are basic cyclic decapeptides. Colistin is polymyxin E and is chemically related to polymyxin B. Colistin is available as the sulfate for oral or topical administration and as the less toxic sulfomethate (colistin methanesulphonate sodium) for parenteral use. Dosages are given in International Units (IU) or met-

ric units depending on the source; 10 units of polymyxin B = 1 µg, 10 units of colistin sulphate or colistin methanesulphonate = 0.5 µg. They are stable, highly water-soluble drugs.

Mechanism of Action

Polymyxins are cationic, surface-active agents that disrupt the structure of cell membrane phospholipids and increase cell permeability by a detergent-like action. This binding is competitive with calcium and magnesium. Polymyxins disorganize the outer membrane of Gram-negative bacteria by binding lipopolysaccharide (LPS, endotoxin) through direct interaction with the anionic lipid A region. This action neutralizes the endotoxin capacity of LPS (Coyne and Fenwick 1993). The bactericidal activity of polymyxin B is concentration-dependent and appears to be related to the ratio of the area under the concentration-time curve to the MIC (AUC:MIC) (Tam et al., 2005).

Antimicrobial Activity

Colistin methanesulphonate has reduced antimicrobial activity compared to colistin sulfate. Polymyxin and colistin are similarly rapidly bactericidal and highly active against many species of Gram-negative organisms, such as *Escherichia coli*, *Salmonella*, and *P. aeruginosa*, but not against *Proteus*, *Serratia*, or *Providencia* (Table 10.1). Veterinary isolates of *P. aeruginosa* are routinely susceptible to polymyxin B (Hariharan et al. 1995). Gram-positive bacteria are resistant. Activity against *P. aeruginosa* is reduced in vivo by the presence of physiologic concentrations of calcium. Susceptible bacteria have an MIC of ≤ 4 µg/ml.

Table 10.1. Activity of polymyxin B and colistin (MIC₉₀, µg/ml) against selected Gram-negative aerobes.

Organism	Polymyxin B MIC ₉₀	Colistin MIC ₉₀
<i>Actinobacillus</i> spp.	0.5	#4
<i>A. pleuropneumoniae</i>	—	1
<i>Bordetella bronchiseptica</i>	0.5	0.12
<i>Brucella canis</i>	100	16-32
<i>Campylobacter jejuni</i>	32	8
<i>Escherichia coli</i>	1	8-16
<i>Histophilus somni</i>	2	0.1
<i>Klebsiella pneumoniae</i>	1	4-8
<i>Pasteurella multocida</i>	4	—
<i>Proteus</i> spp.	128	>128
<i>Pseudomonas aeruginosa</i>	8	8
<i>Salmonella</i> spp.	128	—
<i>Serratia</i> spp.	20	—
<i>Taylorella equigenitalis</i>	2	0.5

Resistance

Acquired resistance is rare but occurs among *P. aeruginosa* as a result of decreased bacterial permeability. There is complete cross-resistance among polymyxins. The mechanism of resistance remains poorly understood. It is believed to result from loss of LPS or replacement of magnesium by protein in the outer membrane. Like the aminoglycosides, first-exposure adaptive resistance occurs (Tam et al., 2005).

Pharmacokinetic Properties

The polymyxins are not absorbed from the gastrointestinal tract. Systemic therapy therefore requires that a parenteral preparation (either polymyxin B sulfate or colistin methanesulphonate sodium) be injected. After absorption from the injection site, polymyxins bind moderately to plasma proteins but extensively to muscle tissue, diffuse poorly through biologic membranes, and attain low concentrations in transcellular fluids and in milk. When administered IV, CSF concentrations of colistin methanesulphonate sodium are 25% of plasma concentrations. Binding to mammalian cell membranes is a significant feature of polymyxin distribution and underlies their accumulation in long-term dosing. They are slowly excreted unchanged by glomerular filtration in the urine. High concentrations of these drugs accumulate in patients with renal insufficiency. The polymyxins are highly

nephrotoxic, causing damage to the renal tubular epithelial cells. For systemic use, colistin methanesulphonate causes less pain at the injection site and less renal toxicity than polymyxin B, but polymyxin B has greater local activity. Methanesulphonate derivatives show better tissue distribution than the bases but are less active and lack the ability to inactivate endotoxin.

Drug Interactions

Polymyxins are synergistic with a variety of antimicrobial drugs through their disorganizing effects on the outer and cytoplasmic membranes. Synergism with sulfonamides and trimethoprim against a variety of Enterobacteriaceae, including otherwise resistant *Proteus* species and *P. aeruginosa*, is recognized. Colistin is synergistic in vivo with rifampin or cef-tazidime against multidrug-resistant *P. aeruginosa* (Giamarellos-Bourboulis et al., 2003).

Toxicity and Adverse Effects

Polymyxins are well tolerated after oral or local administration, but systemic use causes nephrotoxic, neurotoxic, and neuromuscular blocking effects. Colistin is less toxic than polymyxin B, but colistin methanesulphonate has reduced antimicrobial activity compared to colistin sulfate.

In humans, reversible peripheral neuropathy, with paresthesia, numbness around the mouth, blurring of vision, and weakness occur in about 7% of treated patients; neuromuscular blockade causing respiratory insufficiency occurs in about 2% of patients, particularly in those treated with high doses. Nephrotoxicity is commonly observed within 1-4 days of systemic therapy, so renal function should be monitored in all treated patients. Renal function usually returns to normal within 3-9 weeks. Toxicity may be potentiated by other nephrotoxic agents.

Calves treated with 5 mg/kg IM polymyxin B showed lethargy and apathy 2-4 hours after injection, and a small proportion developed transient ataxia. A dose of 5 mg/kg of polymyxin B or its methanesulphonate was highly nephrotoxic, but 2.5 mg/kg had minimal effects. In sheep, 1 of 3 ewes died of respiratory failure within 2 hours of an IM dose of 10 mg/kg polymyxin B (Ziv, 1981). A new formulation of colistin sulfate for IM use, however, showed minimal toxicity in mice, rabbits, and pigs (Lin et al., 2005).

Administration and Dosage

Because of toxicity and the availability of less toxic and more efficacious alternatives, polymyxin has not routinely been used parenterally in animals. For treatment of enteric infections, an oral dose of 5 mg/kg q12 hours has been recommended. For endotoxemia in horses, a dose of polymyxin B at 1000 to 5000 U/kg IV every 8 to 12 hours is suggested (Parviainen et al. 2001).

The usual parenteral dose of colistin methanesulphonate is 3 mg/kg administered IM or IV at 12-hour intervals. A new formulation of colistin sulfate has been recommended for use in piglets at 2.5 mg/kg IM every 12 hours (Lin et al., 2005). For treatment of endotoxic shock in dogs, a dose of 12,500 U/kg every 12 hours is suggested (Senturk, 2005).

Clinical Applications

The low incidence of antimicrobial resistance and their endotoxin-neutralizing properties have renewed interest in the polymyxins, particularly in human medicine. The drugs should not be administered parenterally for more than 5 days unless kidney function is closely monitored. Tissue penetration of polymyxins is poor and is associated with extensive cell membrane binding and inactivation.

The major antimicrobial applications of polymyxins are in the oral treatment of *E. coli* and *Salmonella* diarrheas and in the local treatment of *P. aeruginosa*, such as otitis externa and bacterial keratitis. To widen the range of antimicrobial activity, neomycin and bacitracin are combined with polymyxin B in topical preparations. Neomycin and polymyxin B are also available in a bladder irrigation solution designed for local treatment of *E. coli* cystitis in women.

Cattle

Polymyxins are used in some countries for the treatment of colibacillosis and salmonellosis in calves.

The potential of polymyxins to inactivate endotoxin may be useful in the treatment of coliform mastitis. An IM dose of 5.0 mg/kg of polymyxin B should give milk concentrations exceeding 2 µg/ml for 4 hours, which is sufficient to eliminate the more susceptible coliforms. The anti-endotoxin effect is, however, seen only in the early stages of coliform mastitis, experimentally within 2–4 hours of infusion of endotoxin (Ziv, 1981). Since about 100 µg polymyxin B inactivates only 0.2

µg endotoxin, and endotoxin concentrations may reach 10 µg/ml in coliform mastitis, even intramammary doses are inadequate to eliminate the endotoxin. In an experimental model of coliform mastitis, intramammary infusion of polymyxin B after endotoxin infusion prevented the increase in plasma lactate dehydrogenase activity and moderated the decrease in plasma zinc concentration, but otherwise did not alter the clinicopathologic course of endotoxin-induced acute mastitis (Ziv and Schultze, 1983). Polymyxin B is available in an intramammary mastitis formulation in Canada in combination with penicillin G procaine, novobiocin, dihydrostreptomycin, and hydrocortisone.

Swine

Polymyxins are used in some countries in the oral treatment of neonatal porcine colibacillosis. A new IM injectable formulation of colistin sulfate from China appears promising for the treatment of *E. coli* infections in swine (Lin et al., 2005).

Horses

Polymyxins are used locally to treat bacterial keratitis or metritis caused by otherwise resistant *Klebsiella* spp. or *P. aeruginosa*. Polymyxin B is usually formulated as a "triple antibiotic" ointment or solution, with bacitracin and neomycin.

Polymyxin B has been evaluated for its endotoxin-binding activity in horses. In foals challenged with LPS, it reduces fever, respiratory rate, and serum activities of tumor necrosis factor (TNF) and interleukin-6 (Durando et al., 1994). In adult horses, it ameliorated clinical signs of endotoxemia and decreased plasma TNF activity (Barton et al., 2004). Conversely, polymyxin B was ineffective in ameliorating the endotoxemia associated with carbohydrate overload (Raisbeck et al., 1989). If used, treatment should begin as soon as possible, since the LPS scavenging effects are only beneficial in the first 24–48 hours, after which tolerance to LPS develops. In equine models of endotoxemia, neuromuscular blockade and apnea were not observed, and nephrotoxicity was only observed at very high dosages. Therefore, the anti-endotoxin dose can be administered to horses as a slow IV bolus.

Dogs and Cats

Polymyxins are used in the local treatment of bacterial keratitis, otitis externa, and other skin infections

caused by susceptible Gram-negative bacteria. They are preferred for local *Pseudomonas* infections and may be combined with chlorhexidine or EDTA for synergistic effect. In a canine endotoxemia model, colistin administration improved capillary refill time and hydration and significantly reduced serum TNF concentrations (Senturk, 2005).

Bibliography

- Barton MH, et al. 2004. Polymyxin B protects horses against induced endotoxaemia in vivo. *Equine Vet J* 36:397.
- Coyne CP, Fenwick BW. 1993. Inhibition of lipopolysaccharide-induced macrophage tumor necrosis factor- α synthesis by polymyxin B sulfate. *Am J Vet Res* 54:305.
- Durando MM, et al. 1994. Effects of polymyxin B and *Salmonella typhimurium* antiserum on horses given endotoxin intravenously. *Am J Vet Res* 55:921.
- Giamarellos-Bourboulis EJ, et al. 2003. In vitro interaction of colistin and rifampin on multidrug-resistant *Pseudomonas aeruginosa*. *J Chemother* 15:235.
- Hariharan H, et al. 1995. Antimicrobial drug susceptibility of clinical isolates of *Pseudomonas aeruginosa*. *Can Vet J* 36:166.
- Li J, et al. 2005. Evaluation of colistin as an agent against multi-resistant Gram-negative bacteria. *Int J Antimicrob Agents* 25:11.
- Lin B, et al. 2005. Toxicity, bioavailability and pharmacokinetics of a newly formulated colistin sulfate solution. *J Vet Pharmacol Ther* 28:349.
- Parviainen AK, et al. 2001. Evaluation of polymyxin B in an ex vivo model of endotoxemia in horses. *Am J Vet Res* 62:72.
- Raisbeck MF, et al. 1989. Effects of polymyxin B on selected features of equine carbohydrate overload. *Vet Hum Toxicol* 31:422.
- Senturk S. 2005. Evaluation of the anti-endotoxic effects of polymyxin-E (colistin) in dogs with naturally occurred endotoxic shock. *J Vet Pharmacol Ther* 28:57.
- Tam VH, et al. 2005. Pharmacodynamics of polymyxin B against *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 49:3624.
- Ziv G. 1981. Clinical pharmacology of polymyxins. *J Am Vet Med Assoc* 179:711.
- Ziv G, Schultze WD. 1983. Influence of intramammary infusion of polymyxin B on the clinicopathologic course of endotoxin-induced mastitis. *Am J Vet Res* 44:1446.

Glycopeptides: Vancomycin, Teicoplanin, and Avoparcin

Vancomycin, teicoplanin, and avoparcin are glycopeptide antibiotics with activity against Gram-positive

bacteria and particularly Gram-positive cocci. The drugs inhibit synthesis of cell-wall peptidoglycan by forming bonds with the D-alanyl-D-alanine terminal of muramyl dipeptide. Vancomycin and teicoplanin are currently available for human clinical use in various parts of the world, whereas avoparcin is available for animal agricultural use in some countries. Because of their outstanding activity against a broad-spectrum of Gram-positive bacteria, vancomycin and teicoplanin have often been considered the drugs of "last resort" for serious infections due to drug-resistant Gram-positive pathogens. Glycopeptides had been in clinical use for almost 30 years before high-level resistance, first reported in enterococcal species, emerged. More recently, there have been disturbing reports of low- and intermediate-level resistance to vancomycin in strains of *Staphylococcus aureus*. Avoparcin had been used extensively as a growth promoter for chickens and pigs in Europe. It has been withdrawn for use in Europe because of selection for vancomycin-resistant enterococci in farm animals, which may then be a source of infection for immunosuppressed human patients.

Vancomycin

Chemistry

Vancomycin is a high-molecular-weight glycopeptide, a fermentation product of *Streptomyces orientalis*. It is available as the stable and highly soluble hydrochloride.

Antimicrobial Activity

Vancomycin is bactericidal to most Gram-positive aerobic cocci and bacilli; most Gram-positive aerobes and anaerobes are susceptible but the majority of Gram-negative bacteria are resistant. Organisms with an MIC ≤ 2 –4 $\mu\text{g/ml}$ are regarded as susceptible, those with 8–16 $\mu\text{g/ml}$ as intermediate, and those with ≥ 32 $\mu\text{g/ml}$ as resistant (Table 10.2).

Vancomycin has greatest in vitro activity when the MIC is maintained for the whole dosing interval (Larsson et al., 1996). However, in a murine peritonitis model of *S. pneumoniae* and *S. aureus*, time greater than MIC, C_{max}:MIC, and AUC:MIC were all shown to correlate with efficacy (Knudsen et al., 2000). This suggests that glycopeptide efficacy may be dependent on both time and concentration.

Table 10.2. Activity of vancomycin (MIC₉₀ µg/ml) against selected bacteria.

Organism	MIC ₉₀	Organism	MIC ₉₀
<i>Actinomyces</i> spp.	8	<i>Listeria monocytogenes</i>	1
<i>Arcanobacterium pyogenes</i>	1	<i>Nocardia</i>	256
<i>Clostridium difficile</i>	1	<i>Rhodococcus equi</i>	0.25
<i>C. perfringens</i>	1	<i>Staphylococcus aureus</i>	2
<i>C. septicum</i>	2	Beta-hemolytic streptococci	2
<i>Enterococcus</i> spp.	4		

Resistance

Antibiotic resistance is generally uncommon but occurs with some frequency in *Enterococcus* spp., especially *E. faecium*, in which it has been extensively characterized. VanA encodes resistance to all glycopeptides and is associated with a plasmid-mediated transposable element Tn1546. The VanA gene changes the D-alanyl-D-alanine part of the pentapeptide side chain of N-acetylmuramic acid to D-alanyl-D-lactate, preventing glycopeptide binding and thus evading inhibition of cell wall synthesis. VanB resistance affects vancomycin but not teicoplanin. It is chromosomal in origin and not usually transferable, but acts in a similar manner to VanA. VanC produces a nontransferable lower-level resistance observed in *E. gallinarum*. Cross-resistance may occur among drugs within the glycopeptide class but not with other drug classes. Semisynthetic glycopeptides are being developed to overcome the problem of VanA and VanB resistance.

The world-wide emergence of vancomycin-resistant enterococci (VRE) is a serious human health concern. In the last few years, isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) with reduced susceptibility to glycopeptides have been isolated. Some strains exhibit frank resistance to vancomycin. The recently demonstrated transmission of vancomycin resistance from VRE to MRSA between two human patients in America underscores the potential danger of a coexisting reservoir of both pathogens. There is increasing concern that food and companion animals are a source of these highly resistant pathogens (Prescott et al., 2002; Guardabassi et al., 2004; Hershberger et al., 2005;). Linkage of vancomycin resistance genes with macrolide resistance genes on the same plasmids has been implicated as the cause of VRE persistence in countries that banned the use of avoparacin but con-

tinued the use of tylosin in animal feed (Aarestrup et al., 2001; Boerlin et al., 2001).

Pharmacokinetic Properties

Vancomycin is poorly absorbed after oral administration. Penetration into tissues is relatively poor, although the drug enters CSF when the meninges are inflamed. The half-life in humans is about 6 hours, 2 hours in dogs, and nearly 3 hours in horses (Zaghlol and Brown, 1988; Orsini et al., 1992;). Most of the IV-administered drug is excreted through the kidneys (glomerular filtration), with a small proportion excreted in bile. Vancomycin hydrochloride causes marked tissue damage, so it is administered by IV infusion over a 60-minute period. Dosage adjustment is required for patients with renal impairment. Plasma concentrations can be monitored and dose intervals adjusted to give trough concentrations approximating the MIC of susceptible organisms.

Drug Interactions

Vancomycin is synergistic with aminoglycosides against Gram-positive cocci. It appears to be synergistic in vivo with rifampin against *Staphylococcus aureus*. Vancomycin is antagonistic in vitro with many other drugs, so it must be carefully administered.

Toxicity and Adverse Effects

Vancomycin is highly irritating to tissues on injection and must be administered slowly IV in dilute form. Rapid IV injection produces a histamine-like reaction in humans (red-neck syndrome). The drug is ototoxic in humans, particularly in patients treated with large doses or in those with renal insufficiency. Recent purer forms of the drug, as well as lower doses, have been associated with lower risk of ototoxicity. The drug is also potentially nephrotoxic, an adverse effect compounded by high doses and concurrent use of nephrotoxic drugs. There is no information on toxicity in domestic animals.

Administration and Dosage

Dosage recommendations are largely empirical. For treating intestinal disease, 5–10 mg/kg PO every 12 hours has been recommended. In dogs, Zaghlol and Brown (1988) recommended a parenteral dosage of 15 mg/kg q6 hours. In horses, Orsini and others (1992) recommended 4.3–7.5 mg/kg given as a 1-hour IV infusion every 8 hours. It was dosed in a cat at 19.4

mg/kg every 12 hours for 10 days (Pressel et al. 2005). Vancomycin can be formulated as antimicrobial-impregnated polymethylmethacrylate (AIPMM) beads or in bone cement for local therapy of musculoskeletal infections (Liu et al., 2002; Joosten et al., 2005).

Clinical Applications

There are few indications for the use of vancomycin in animals, particularly since this is a "last resort" drug in human medicine. In humans, it is primarily used to treat infections caused by multiresistant Gram-positive bacteria that cannot be treated with other drugs. It may be used to treat patients allergic to penicillins and cephalosporins. It is also the drug of choice in people for the oral treatment of *Clostridium difficile* colitis because of its activity and narrow bactericidal spectrum.

There are few reports of the clinical use of vancomycin in veterinary medicine. The decision to use vancomycin to treat a highly resistant pathogen in a veterinary patient should include consideration of the health risks to others, both human and animal, in contact with the patient. An effective infection control program is mandatory for such cases. Vancomycin therapy resolved clinical signs of cholangiohepatitis in cats, but did not necessarily produce a microbiological cure (Jackson et al., 1994; Pressel et al., 2005). Vancomycin AIPMM beads were used in conjunction with systemic vancomycin therapy at 6 mg/kg IV every 8 hours in a post-surgical infection with methicillin-resistant *Staphylococcus epidermidis* in a horse (Trostle et al., 2001).

Bibliography

- Aarestrup FM, et al. 2001. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. *Antimicrob Agents Chemother* 45:2054.
- Boerlin P, et al. 2001. Antimicrobial growth promoter ban and resistance to macrolides and vancomycin in enterococci from pigs. *J Clin Microbiol* 39:4193.
- Guardabassi L, et al. 2004. Pet animals as reservoirs of antimicrobial-resistant bacteria. *J Antimicrob Chemother* 54:321.
- Hershberger E, et al. 2005. Epidemiology of antimicrobial resistance in enterococci of animal origin. *J Antimicrob Chemother* 55:127.
- Jackson MW, et al. 1994. Administration of vancomycin for treatment of ascending bacterial cholangiohepatitis in a cat. *J Am Vet Med Assoc* 204:602.
- Joosten U, et al. 2005. Effectiveness of hydroxyapatite-vancomycin bone cement in the treatment of *Staphylococcus aureus* induced chronic osteomyelitis. *Biomaterials* 26:5251.
- Knudsen JD, et al. 2000. Pharmacodynamics of glycopeptides in the mouse peritonitis model of *Streptococcus pneumoniae* or *Staphylococcus aureus* infection. *Antimicrob Agents Chemother* 44:1247.
- Larsson AJ, et al. 1996. The concentration-independent effect of monoexponential and biexponential decay in vancomycin concentrations on the killing of *Staphylococcus aureus* under aerobic and anaerobic conditions. *J Antimicrob Chemother* 38:589.
- Liu SJ, et al. 2002. In vivo release of vancomycin from biodegradable beads. *J Biomed Mater Res* 63:807.
- Orsini JA, et al. 1992. Vancomycin kinetics in plasma and synovial fluid following intravenous administration in horses. *J Vet Pharmacol Therap* 15:351.
- Prescott JE, et al. 2002. Antimicrobial drug use and resistance in dogs. *Can Vet J* 43:107.
- Pressel MA, et al. 2005. Vancomycin for multi-drug resistant *Enterococcus faecium* cholangiohepatitis in a cat. *J Feline Med Surg* 7:317.
- Trostle SS, et al. 2001. Treatment of methicillin-resistant *Staphylococcus epidermidis* infection following repair of an ulnar fracture and humeroradial joint luxation in a horse. *J Am Vet Med Assoc* 218:554.
- Zaghlool HA, Brown SA. 1988. Single- and multiple-dose pharmacokinetics of intravenously administered vancomycin in dogs. *Am J Vet Res* 49:1637.

Teicoplanin

Teicoplanin has a molecular structure similar to that of vancomycin and is also a derivative of an actinomycete. It is a complex of five closely related antibiotics.

Teicoplanin has activity similar to and slightly greater than vancomycin, being restricted also in activity to Gram-positive bacteria. It has excellent activity against *S. aureus* (including methicillin-resistant strains) and against streptococci (in which it is more active than vancomycin), *L. monocytogenes*, *C. difficile*, *C. perfringens*, and other Gram-positive bacteria. *Enterococcus faecalis* are somewhat less sensitive than other cocci. *Nocardia* are resistant. Susceptible organisms are those with an MIC ≤ 4 μ /ml. Teicoplanin is usually bactericidal to organisms with MIC ≤ 16 μ g/ml. Activity in vitro is more affected by test conditions than the activity of vancomycin.

Like vancomycin, development of resistance to teicoplanin is uncommon and, in fact, these drugs were at one time optimistically regarded as resistance-resistant. Nevertheless, VanA resistance (causing cross-resistance to teicoplanin) occurs in enterococci and re-

sistance may develop in coagulase-negative staphylococci, either as a result of selection of mutants with progressive increases in MIC occurring in bacteria during treatment or, less commonly, by plasmid-mediated mechanisms.

Teicoplanin is not absorbed after oral administration. Absorption after IM injection is excellent and the drug distributes widely into tissues in extracellular fluid. The half-life is remarkably prolonged in humans, between 45 and 70 hours after IV injection, but no pharmacokinetic studies have been done in animals. Penetration into cerebrospinal fluid is poor because of high molecular weight and poor lipid solubility. Elimination is almost entirely renal.

Teicoplanin is synergistic with aminoglycosides against some Gram-positive cocci, including penicillin-tolerant enterococci, is indifferent or additive with rifampin, and may be synergistic with imipenem against Gram-positive cocci.

In humans, teicoplanin is usually well tolerated. Adverse effects reported, in order of frequency, include: hypersensitivity skin reactions (rash, pruritus, urticaria), pain (IM) or phlebitis (IV) at injection sites, and rarely nephrotoxicity or ototoxicity (usually in patients also receiving aminoglycosides). Teicoplanin, unlike vancomycin, can be administered by rapid IV injection. No information on toxicity in domestic animals is available.

Teicoplanin is used in human medicine for the treatment of serious infections caused by Gram-positive bacteria where a bactericidal drug is needed, where there is resistance to alternative drugs, or where synergism with an aminoglycoside for broad-spectrum or enhanced activity is required. Uses include septicemia, endocarditis, bone and joint infections, and cystitis caused by multiresistant enterococci.

Bibliography

- Campoli-Richards DM, et al. 1990. Teicoplanin: A review of its antibacterial activity, pharmacokinetic properties and therapeutic potential. *Drugs* 40:449.
- Cercenado E, et al. 1996. Emergence of teichoplanin-resistant coagulase-negative staphylococci. *J Clin Microbiol* 34:1765.
- Dutka-Malen S, et al. 1990. Phenotypic and genotypic heterogeneity of glycopeptide resistance determinants in Gram-positive bacteria. *Antimicrob Agents Chemother* 34:1875.

Avoparcin

Avoparcin was used extensively as a growth promoter in chickens and pigs in Europe. The recognition that it selected for VRE in animals, and in a high proportion of meat products derived from these animals, led to its withdrawal from use in Denmark and subsequently in all of Europe (Casewell et al., 2003). The importance of vancomycin-resistant enterococci (VREs) is that, because of the inherent resistance of many enterococci to antibiotics, these may cause untreatable or difficult-to-treat infections in high risk patients (e.g., neutropenic cancer patients) treated with broad-spectrum antibiotics. With the avoparcin ban in Denmark in 1995, there was an immediate decrease in VRE isolated from poultry, but not in pigs until tylosin was also banned from feed use (Aarestrup et al., 2001). However, VREs have continued to cause significant problems in human hospitals not only in Europe but also in North America, where avoparcin has never been used in animals. Recent studies conclude that animal-associated VRE probably reflect the former use of avoparcin in animal production, whereas VRE in human-associated samples may be a result of antibiotic use in hospitals (Kuhn et al., 2005).

Bibliography

- Aarestrup FM, et al. 2001. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. *Antimicrob Agents Chemother* 45:2054-2059.
- Casewell M, et al. 2003. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *J Antimicrob Chemother* 52:159.
- Kuhn I, et al. 2005. Occurrence and relatedness of vancomycin-resistant enterococci in animals, humans, and the environment in different European regions. *Appl Environ Microbiol* 71:5383.

Bacitracin

Bacitracin is a polypeptide product of *Bacillus subtilis* that inhibits the formation of bacterial cell-wall peptidoglycan by complexing directly with the pyrophosphate carrier and inhibiting the dephosphorylation reaction required for its regeneration. It is bactericidal to Gram-positive bacteria but has little activity against

Gram-negative organisms. Resistance develops slowly and is rare. One unit = 26 µg of the USP standard.

Because bacitracin is highly nephrotoxic after parenteral administration, it is generally only used in the topical treatment of superficial infections of the skin and mucosal surfaces, particularly where activity against *S. aureus* is required. Because beta-lactams are potent contact sensitizers, they are not administered topically, so bacitracin replaces them for Gram-positive coverage in topical products. It is often combined with neomycin and polymyxin B for broad-spectrum activity in treating minor skin wounds or bacterial keratitis. In a review of bacterial keratitis, only 64% of *S. zooepidemicus* isolates were sensitive to bacitracin (Keller and Hendrix, 2005). It is administered orally for growth promotion in poultry and swine, and in the prevention and treatment of enteritis caused by *C. perfringens* (e.g., in type C enteritis in suckling pigs or necrotic enteritis in poultry) (Brennan et al., 2003). It is not effective in the treatment of swine dysentery. Incorporation in feed may prevent proliferative adenomatosis in swine, although *Lawsonia intracellularis* is resistant in vitro (Kyriakis et al., 1996). It might be useful in preventing *C. spiroforme* enteritis in rabbits and *C. difficile* diarrhea in horses, although one study reported complete resistance by equine *C. difficile* isolates to this drug (Jang et al., 1997).

Bibliography

- Brennan J, et al. 2003. The efficacy of bacitracin methylene disalicylate when fed in combination with narasin in the management of necrotic enteritis in broiler chickens. *Poult Sci* 82:360.
- Jang SS, et al. 1997. Antimicrobial susceptibilities of equine isolates of *Clostridium difficile* and molecular characterization of metronidazole-resistant strains. *Clin Infect Dis* 25:S266.

Keller RL, Hendrix DV. 2005. Bacterial isolates and antimicrobial susceptibilities in equine bacterial ulcerative keratitis (1993-2004). *Equine Vet J* 37:207.

Kyriakis SC, et al. 1996. Clinical evaluation of in-feed zinc bacitracin for the control of porcine intestinal adenomatosis in growing/fattening pigs. *Vet Rec* 138:489.

Fosfomycin

Fosfomycin (L-[*cis*]-1,2 epoxypropyl phosphonic acid) is a phosphoenolpyruvate analogue which irreversibly inhibits pyruvyl transferase, the enzyme catalysing the first step of peptidoglycan biosynthesis. It is produced by various *Streptomyces* spp. It is available in some countries for human and veterinary use, but not in North America. It is particularly active against many Enterobacteriaceae including *E. coli* in the 1–8 µg/ml MIC range; *P. aeruginosa* is resistant. Activity against Gram-positive bacteria such as *S. aureus*, enterococci and streptococci is in the 2–64 µg/ml range. Activity is reduced by alkaline pH and the presence of glucose, sodium chloride or phosphates in culture media. Resistance, which can be chromosomal or plasmid-mediated, is said to be uncommon. There is no cross-resistance with other antibacterial drugs. Adverse effects are usually minor gastrointestinal disturbances. Different forms of the drug are available for oral or parenteral use.

Bibliography

- Aramayona JJ, et al. 1997. Pharmacokinetics of fosfomycin in chickens after a single intravenous dose and tissue levels following chronic oral administration. *Vet Res* 28:581.
- Patel SS, et al. 1997. Fosfomycin tromethamine. *Drugs* 53:637.
- Stein GE. 1998. Single-dose treatment of acute cystitis with fosfomycin tromethamine. *Annals Pharmacother* 32:215.

Lincosamides, Pleuromutilins, and Streptogramins

Steeve Giguère

Lincosamides, pleuromutilins, and streptogramins are structurally distinct but share many common properties. They are basic compounds characterized by high lipid solubility, wide distribution in the body, and capacity to penetrate cellular barriers. In addition, they have a common site of action on the 50S ribosome.

Lincosamides: Lincomycin, Clindamycin and Pirlimycin

Chemistry

Lincomycin, the parent compound, was isolated in 1962 from the fermentation of *Streptomyces lincolnensis* subsp. *lincolnensis*. Many modifications of the lincomycin molecule have been developed in an attempt to produce an improved antibiotic. Of these, only clindamycin showed distinct advantages over lincomycin. Pirlimycin, a clindamycin analog, is also approved as an intramammary infusion for the treatment of mastitis in cattle. A fourth molecule, mirincamycin, is currently undergoing clinical development in human medicine. The chemical structures of lincomycin and clindamycin are displayed in Figure 11.1.

Mechanism of Action

The lincosamides inhibit protein synthesis by binding to the 50S ribosomal subunit and inhibiting peptidyl transferases. The ribosomal binding sites are the same as or closely related to those that bind macrolides, streptogramins, and chloramphenicol. Lincosamides can be bactericidal or bacteriostatic, depending on the drug concentration, bacterial species, and inoculum of bacteria. They are primarily active against Gram-positive bacteria and most obligate anaerobes. Most

aerobic and facultative anaerobic Gram-negative bacteria are intrinsically resistant because of impermeability and methylation of the ribosomal binding site of lincosamides.

Antimicrobial Activity

Lincosamides are moderate-spectrum antimicrobial drugs. Clindamycin is active against many Gram-positive bacteria, Gram-positive and Gram-negative anaerobic bacteria, and some mycoplasma (Table 11.1). Clindamycin is several times more active than lincomycin, especially against anaerobes and *Staphylococcus* spp. Both drugs lack activity against most aerobic or facultative Gram-negative bacteria.

Good susceptibility ($MIC \leq 2 \mu\text{g/ml}$). Gram-positive aerobes: *Bacillus* spp., *Corynebacterium* spp., *Erysipelothrix rhusiopathiae*, staphylococci, streptococci (but not enterococci). Gram-negative bacteria: *Campylobacter jejuni*. Anaerobes: many anaerobes including *Actinomyces* spp., *Bacteroides* spp. (including *B. fragilis*), and *C. perfringens* (but not all other *Clostridium* spp.). *Fusobacterium* spp. and anaerobic cocci are particularly susceptible to clindamycin. Activity of clindamycin against anaerobes is similar to chloramphenicol and metronidazole. Clindamycin has activity against some protozoa such as *Toxoplasma gondii* and *Plasmodium falciparum*. It has some activity against *Pneumocystis carinii*.

Resistant ($MIC \geq 4 \mu\text{g/ml}$). All aerobic and facultative Gram-negative rods (e.g., Enterobacteriaceae), *Nocardia* spp., and *Mycobacterium* spp. Lincosamides are also inactive against *Enterococcus faecalis* and *E. faecium*.

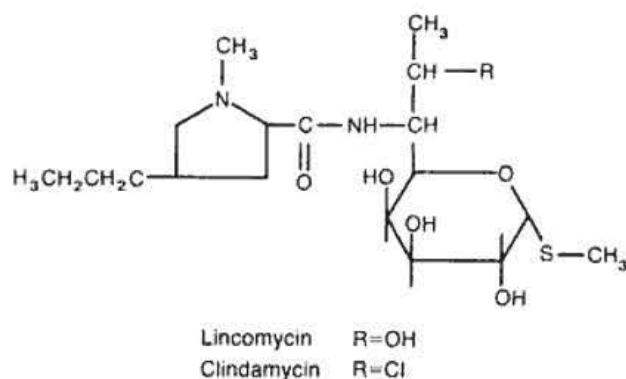


Figure 11.1. Structural formulas of lincomycin and clindamycin.

Resistance

Resistance can develop to the lincosamides alone, but more commonly cross-resistance occurs among macrolides, lincosamides, and streptogramin group B antibiotics (MLSB resistance). Resistance is the result of methylation of adenine residues in the 23S ribosomal RNA of the 50S ribosomal subunit, which prevents drug binding to the target site. Different bacterial species are able to synthesize an enzyme, encoded by a series of structurally related erythromycin-resistant methylase (*erm*) genes. This cross-resistance is of two types: (1) constitutive resistance (MLSB_c), where bacteria show high-level resistance to all MLSB antibiotics, and (2) dissociated inducible cross-resistance (MLSB_i), in which bacteria resistant to macrolides but initially fully susceptible to clindamycin rapidly develop resistance to lincosamides when exposed to macrolides. Constitutive resistant mutants are rapidly selected from the inducible strains during treatment with either lincosamides or macrolides. Constitutive resistance may be more common among bacteria isolated from food animals fed tylosin or virginiamycin as growth promoters. MLSB_c isolates are readily recognized during in vitro susceptibility testing as being resistant to both macrolides and clindamycin. The problem is that MLSB_i resistance is not detected by standard in vitro susceptibility testing methods. Such isolates appear resistant to macrolides but susceptible to clindamycin under standard testing conditions. As a result, isolates that are resistant to macrolides but susceptible to clindamycin should also be tested for methylase-mediated clindamycin resistance by an additional assay, the D-zone test. Isolates that demonstrate inducible clindamycin resistance

Table 11.1. In vitro activity (MIC₉₀) of lincosamides and pleuromutilin antibiotics (μg/ml) against selected bacterial and mycoplasmal pathogens.

Organisms	Clindamycin/ lincomycin ^a	Pirlimycin	Tiamulin
Gram-positive aerobes			
<i>Arcanobacterium pyogenes</i>	<0.06 ^b	—	0.03
<i>Erysipelothrix rhusiopathiae</i>	1	—	4
<i>Rhodococcus equi</i>	4	—	64
<i>Staphylococcus aureus</i>	0.25	1	0.03
<i>Streptococcus equi</i>	16	8	0.5
<i>S. agalactiae</i>	4	0.5	—
<i>S. dysgalactiae</i>	16	1	—
<i>S. uberis</i>	>32	8	—
<i>Enterococcus faecalis</i>	16	2	>32
Gram-negative aerobes			
<i>Actinobacillus pleuropneumoniae</i>	>32 ^a	—	8
<i>Histophilus somni</i>	—	—	2
<i>Mannheimia haemolytica</i>	—	—	4
<i>Pasteurella multocida</i>	>25	—	32
<i>Escherichia coli</i>	>32	>32	32
<i>Klebsiella spp.</i>	>32	>32	>128
<i>Enterobacter spp.</i>	>32	>32	>32
Anaerobes			
<i>Dichelobacter nodosus</i>	0.25	—	—
<i>Bacteroides fragilis</i>	0.5	0.06	—
<i>Fusobacterium necrophorum</i>	0.5	0.5	0.016
<i>Brachyspira hyodysenteriae</i>	4	—	0.25
<i>Brachyspira pilosicoli</i>	8	—	0.5
<i>Clostridium perfringens</i>	4	0.5	—
Mycoplasma			
<i>Mycoplasma bovis</i>	>256	—	0.25
<i>M. hyorhinis</i>	2 ^a	—	0.25
<i>M. hyopneumoniae</i>	0.12 ^a	—	0.25
<i>M. hyosynoviae</i>	—	—	0.06
<i>M. mycoides mycoides</i>	—	—	0.5
<i>Ureaplasma spp.</i>	—	—	0.06
Other			
<i>Leptospira spp.</i>	0.2	—	4
<i>Lawsonia intracellularis</i>	32 ^a	—	4

^aMIC values for lincomycin; all others are for clindamycin.

^bSome reports show resistance to clindamycin.

based on a D-zone test should be reported as clindamycin resistant (Lewis and Jorgensen, 2005).

As well as this broad-spectrum of resistance, resistance specific to lincosamides has been reported. Staphylococci, streptococci and enterococci can owe their resistance to lincosamides to enzymatic inactivation of these drugs. These enzymes induce phosphorylation and nucleotidation of the hydroxyl group at position 3 of lincosamide molecules.

Pharmacokinetic Properties

Lincosamides are basic compounds with pK_a values of about 7.6. They have high lipid solubility and consequently large apparent volumes of distribution. They are well absorbed from the intestines of nonherbivores and eliminated mainly by hepatic metabolism, although about 20% is eliminated in active form in the urine. Tissue concentrations consistently exceed serum concentrations severalfold because of passage across cell membranes. Because of the lincosamide's basic character, ion trapping also occurs in tissues, such as the udder and prostate, where pH is lower than blood. Extensive binding to plasma proteins and relatively rapid elimination prevent concentrations in cerebrospinal fluid (CSF) from exceeding 20% of serum concentrations. Clindamycin achieves effective concentrations in bone, although levels are relatively low, perhaps 10–20% of serum concentrations.

Drug Interactions

Combination with spectinomycin appears to give marginally enhanced activity against mycoplasmas in vitro. Clindamycin is commonly combined with an aminoglycoside or a fluoroquinolone in human medicine to treat or prevent mixed aerobic-anaerobic bacterial infections, particularly those associated with intestinal spillage into the peritoneum. The combination generally has additive or synergistic effects in vitro against a wide range of bacteria. Clindamycin has synergistic effects with metronidazole against *B. fragilis* but only additive effects with trimethoprim-sulfamethoxazole combination against common Gram-negative or Gram-positive aerobes. Combination with macrolides or chloramphenicol is antagonistic in vitro.

Toxicity and Adverse Effects

The major toxic effect of the lincosamides is their ability to cause serious and fatal diarrhea in humans, horses, rabbits, and other herbivores.

In humans, mild diarrhea follows the use of lincosamides in up to 10% of patients, but in some (up to 2.5% of those treated) this may become severe, resulting in pseudomembranous colitis with profound shock, dehydration, and death. The disease is caused by the rapid colonic growth of lincosamide-resistant *Clostridium difficile* through destruction of competing anaerobic microflora of the colon. Treatment with vancomycin or metronidazole is often successful. Less

serious toxic effects in humans include depressed neuromuscular transmission and post-anesthetic paralysis, depression of cardiac muscle after rapid IV injection, mild liver damage, drug rashes, and urticaria.

In cattle, oral administration of lincomycin at concentrations as low as 7.5 parts/million (ppm) in feed has resulted in inappetence, diarrhea, ketosis, and decreased milk production. Inadvertent contamination of feed with 8–10 ppm of lincomycin and 40 ppm of metronidazole caused some affected cows to develop severe diarrhea and to lose consciousness (Lang, 1979). In horses, lincosamides administered by the parenteral or oral route can cause severe enterocolitis, which may be fatal. In one inadvertent mixing of lincomycin in horse feed, a dose of 0.5 mg/kg caused an outbreak of diarrhea in which one horse died (Raisbeck et al., 1981). Anal swelling, diarrhea, irritable behavior, and skin reddening have been reported in pigs, but these signs are generally self-limiting within 5–8 days.

Lincosamides are highly toxic to rabbits, guinea pigs, and hamsters. Concentrations as low as 8 ppm accidentally added to feed have been followed by severe and fatal cecocolitis in rabbits. This effect is the result of bacterial overgrowth in the large bowel of *C. difficile* or *Clostridium spiroforme* (Borriello and Carman, 1983).

Lincomycin is relatively nontoxic to dogs and cats. Anorexia, vomiting and diarrhea have sometimes occurred following oral administration. Anaphylactic shock has been reported after IM injection. Because of their peripheral neuromuscular blocking and cardiac depressive effects, lincosamides should not be given with anesthetics or by rapid IV injection. Clindamycin given IM is very painful.

Administration and Dosage

Usual dosages are shown in Table 11.2.

After oral administration to monogastric animals, lincomycin is generally absorbed well and clindamycin is absorbed almost completely. Food significantly reduces absorption of both drugs, especially lincomycin. Complete absorption occurs from IM injection sites. Clindamycin palmitate, available as a syrup for oral administration, is rapidly hydrolyzed in the intestine before absorption. The drug is also available in capsules as the hydrochloride for oral administration and as the phosphate for IM, SC, or IV injection. The SC route is superior to the IM route in terms of local tolerance and serum concentrations. Lincomycin is avail-

Table 11.2. Usual dosages of lincosamides and pleuromutilins in animals.

Species	Drug	Dosage (mg/kg)	Route	Interval (h)
Dog/cat	Clindamycin	5-11	PO, IV, IM, SC	12-24
	Lincomycin	10-20	PO, IV, IM	12-24
Ruminants	Lincomycin	5-10	IM	12-24
	Tiamulin	20	IM	24
Swine	Lincomycin	10	IM	24
	Tiamulin	10-15	IM	24
		8-23	PO, feed	24
	Valnemulin	1.5-4	PO, feed	24

able as the hydrochloride for PO, IM, and IV administration. The dosage should be reduced in patients with hepatic insufficiency.

Clinical Applications

Lincosamides are used in the treatment of staphylococcal infections (dermatitis, osteomyelitis) caused by penicillin G-resistant organisms, for other Gram-positive aerobic infections in penicillin-sensitive individuals, and in the treatment of anaerobic infections. In general, clindamycin is preferred to lincomycin. Clindamycin has excellent activity against anaerobes, equivalent to alternatives such as cefoxitin, chloramphenicol, and metronidazole. Clindamycin may be combined with an aminoglycoside or a fluoroquinolone in the treatment of mixed aerobic/anaerobic infections. Clindamycin may be preferable to penicillin G or ampicillin in the treatment of streptococcal toxic shock syndrome, since it better inhibits superantigen synthesis (Sriskadan et al., 1997). Lincosamides penetrate well into the prostate and eyes. There are some doubts about the efficacy in vivo of clindamycin in the treatment of toxoplasmosis, although combination with pyrimethamine may enhance efficacy. Clindamycin may be useful in treating *Pneumocystis carinii* infection, in combination with primaquine. In swine, lincomycin is used extensively in the prevention and treatment of dysentery and sometimes for mycoplasma infections.

Cattle, Sheep, and Goats

Lincomycin-spectinomycin combinations are sometimes used in the parenteral treatment of respiratory disease in cattle because lincomycin inhibits mycoplasma, *Arcanobacterium pyogenes*, and anaerobic bacteria, and spectinomycin is often active against

Mannheimia and *Histophilus somni* (*Haemophilus somnus*). A field trial has confirmed the value of the combination (Pobel et al., 1997). Lincomycin (8 g/L) administered as a spray once daily for 5 days was effective in the control of papillomatous digital dermatitis in cattle (Shearer and Elliott, 1998). A single IM injection of the combination (5 mg/kg lincomycin, 10 mg/kg spectinomycin) cured over 90% of sheep with acute or chronic foot rot and was almost as effective as the same dose given on each of 3 days (Venning et al., 1990). The combination has also been used in the treatment of rams to prevent ureaplasma contamination of semen (Marcus et al., 1994).

Lincomycin and clindamycin can be used in the parenteral treatment of acute staphylococcal or streptococcal mastitis at a dosage of 10 mg/kg q24 hours. The drugs can be given by the intramammary route for the same purpose. Lincomycin has been used parenterally in sheep and cattle. Successful parenteral lincomycin treatment of arthritis and pedal osteomyelitis usually associated with *A. pyogenes* was reported by Plenderleith (1988). The catastrophic effects of oral administration of lincomycin to sheep were described by Bulgin (1988).

The major indication for the use of lincosamides in cattle is intramammary infusion in cases of mastitis. Pirlimycin is commercially available for that purpose. Intramammary pirlimycin has been proven effective against mastitis caused by *Staphylococcus* species such as *Staphylococcus aureus* and *Streptococcus* species such as *Streptococcus dysgalactiae* and *Streptococcus uberis* (Gillespie et al., 2002; Olivier et al., 2004).

Swine

Lincomycin is largely used in pigs to control dysentery and *Mycoplasma* infections; control of erysipelas and

streptococcal infections may be incidental benefits to incorporating the drug in feed for the principal purposes. Lincomycin is used in feed or water (33 mg/L) to treat (100 ppm feed) or prevent (40 ppm feed) swine dysentery; lincomycin can be administered 11 mg/kg IM for 3–7 days. A drawback has been failure to sterilize *B. hyodysenteriae*, so that withdrawal of drug is followed by recrudescence of infection. Nevertheless, whole-herd medication has apparently eradicated swine dysentery from closed herds, even in some cases of infection with apparently resistant organisms. Lincomycin is effective in reducing losses from *Mycoplasma hyosynoviae* and *Mycoplasma hyorhinis*. Pleuromutilins are considerably more effective than lincomycin in control of swine dysentery and *Mycoplasma* infections in swine. Lincomycin delivered in the drinking water has also been effective for the treatment of proliferative enteropathy, both in a field study and following experimental infection (Bradford et al., 2004). Lincomycin may be given in feed, water, or by IM injection.

Horses

Lincomycin and clindamycin have been used experimentally to induce enterocolitis in horses. These drugs should not be used in horses, although there are reports of apparently successful use in the treatment of osteomyelitis by IM injection without toxic effects (Plenderleith et al., 1988).

Dogs and Cats

Lincosamides are used in the treatment of abscesses, osteomyelitis, periodontal disease, and soft tissue or wound infections that involve Gram-positive cocci or anaerobic bacteria. In experimentally induced *Staphylococcus aureus* osteomyelitis in dogs, a dosage of 11 mg/kg clindamycin administered q12 hours for 28 days effectively resolved the infection (Braden et al., 1988). Dosage of 5.5 mg/kg q12 hours was less effective. Clindamycin has been administered at low daily oral dosage in the successful prophylaxis of recurrent staphylococcal skin infections (Klempner and Styrt, 1988). Field trials have demonstrated the 94–100% efficacy of once-daily dosing with 11 mg/kg orally (average duration 45 days) in the treatment of deep pyoderma in dogs (Harvey et al., 1993; Scott et al., 1998). Lincomycin (22 mg/kg, q12 hours) orally is equally effective in the treatment of staphylococcal skin disease in dogs (Harvey et al., 1993).

In a study of experimental anaerobic infections in dogs, Berg et al. (1984) found that clindamycin, 5.5 or 11 mg/kg administered twice daily IM, was highly efficacious and gave better results than lincomycin, 22 mg/kg twice daily. Clindamycin is used effectively in the treatment of dental infections in dogs, when combined with dental surgery or cleaning (Johnson et al., 1992). Anecdotally, its routine use in periodontal surgery has been associated with problems of salmonellosis in veterinary hospitals. Clindamycin is useful for prostatic infections caused by Gram-positive bacteria. Dosing of 11 mg/kg once daily orally appears to be appropriate, but the same dose could be administered twice daily in serious infections (e.g., osteomyelitis).

Clindamycin has been used successfully in the treatment of toxoplasmosis in a dog and in cats, although it was unsuccessful in treating feline chorioretinitis or anterior uveitis in all cases (Greene et al., 1985; Lappin et al., 1989). Clindamycin administered to cats experimentally infected with toxoplasmosis exacerbated disease, increasing morbidity and mortality from generalized disease (Davidson et al., 1996). This adverse effect was attributed to an effect of the drug in decreasing phagocyte activity. Combination with pyrimethamine was less effective in long-term treatment of toxoplasmic encephalitis in human patients than the combination of pyrimethamine-sulfadiazine (Katlama et al., 1996). Clindamycin was successful in resolving dermatitis caused by *Neosporium caninum* in a dog (Dubey et al., 1995). Clindamycin was also successful for the treatment of dogs experimentally infected with *Babesia gibsoni* (Wulansari et al., 2003).

Poultry

Lincomycin-spectinomycin combination is administered orally to young chickens for the control of mycoplasmal air sacculitis and complicated chronic respiratory disease caused by *M. gallisepticum*. Lincomycin has also been used in the control of necrotic enteritis caused by susceptible pathogens such as *C. perfringens*.

Bibliography

- Berg JN, et al. 1984. Clinical models for anaerobic bacteria: Infections in dogs and their use in the testing of the efficacy of clindamycin and lincomycin. *Am J Vet Res* 45:1299.
- Boothe DM, et al. 1996. Plasma disposition of clindamycin microbiological activity in cats after single oral doses of

- clindamycin hydrochloride as either capsules or aqueous solution. *J Vet Pharm Ther* 19:491.
- Borriello SP, Carman RJ. 1983. Association of iota-toxin and *Clostridium spiroforme* with both spontaneous and antibiotic-associated diarrhea and colitis in rabbits. *J Clin Microbiol* 17:414.
- Braden TD, et al. 1988. Posologic evaluation of clindamycin, using a canine model of post-traumatic osteomyelitis. *Am J Vet Res* 48:1101.
- Bradford JR, et al. 2004. Evaluation of lincomycin in drinking water for treatment of induced porcine proliferative enteropathy using a swine challenge model. *Vet Ther* 5:239.
- Brown SA, et al. 1990. Tissue concentrations of clindamycin after multiple oral doses in normal cats. *J Vet Pharm Ther* 13:270.
- Bulgin MS. 1988. Losses related to the ingestion of lincomycin-medicated feed in a range sheep flock. *J Am Vet Med Assoc* 192:1083.
- Davidson MG, et al. 1996. Paradoxical effect of clindamycin in experimental acute toxoplasmosis in cats. *Antimicrob Agents Chemother* 40:1352.
- Dubey JP, et al. 1995. Canine cutaneous neosporosis: clinical improvement with clindamycin. *Vet Dermatol* 6:37.
- Gillespie BE, et al. 2002. Efficacy of extended pirlimycin hydrochloride therapy for treatment of environmental *Streptococcus* spp. and *Staphylococcus aureus* intramammary infections in lactating dairy cows. *Vet Ther* 3:373.
- Greene CG, et al. 1985. Clindamycin for treatment of toxoplasma polymyositis in a dog. *J Am Vet Med Assoc* 187:631.
- Harvey RG, et al. 1993. A comparison of lincomycin hydrochloride and clindamycin hydrochloride in the treatment of superficial pyoderma in dogs. *Vet Rec* 132:351.
- Johnson LA, et al. 1992. Klinische wirksamkeit von clindamycin (Cleorobe) bei infektionen des zahn-, mund- und kieferbereiches des hundes. *Prakt Tier* 73:94.
- Katlama C, et al. 1996. Pyrimethamine-clindamycin vs. pyrimethamine-sulfadiazine as acute and long-term therapy for toxoplasmic encephalitis in patients with AIDS. *Clin Infect Dis* 22:268.
- Klempner MS, Styrt B. 1988. Prevention of recurrent staphylococcal skin infections with low-dose oral clindamycin therapy. *J Am Med Assoc* 260:2682.
- Lang DC. 1979. Inadvertent inclusion of antibiotics in cattle cake. *Vet Rec* 104:173.
- Lappin MR, et al. 1989. Clinical feline toxoplasmosis. Serologic diagnosis and therapeutic management of 15 cases. *J Vet Intern Med* 3:139.
- Lewis JS, Jorgensen JH. 2005. Inducible clindamycin resistance in staphylococci: should clinicians and microbiologists be concerned. *Clin Infect Dis* 40:280.
- Marcus S, et al. 1994. Lincomycin and spectinomycin in the treatment of breeding rams with semen contaminated with ureaplasmas. *Res Vet Sci* 57:393.
- Oliver SP, et al. 2004. Influence of prepartum pirlimycin hydrochloride or penicillin-novobiocin therapy on mastitis in heifers during early lactation. *J Dairy Sci* 87:1727.
- Plenderleith RJW. 1988. Treatment of cattle, sheep and horses with lincomycin: case studies. *Vet Rec* 122:112.
- Pobel T, et al. 1997. Comparaison de l'administration quotidienne ou biquotienne de la spectinomycine (Spectam G. A.) dans le traitement des infections respiratoires a *Pasteurella haemolytica* ou *Pasteurella multocida* associes ou non a *Mycoplasma bovis*. *Revue Med Vet* 148:47.
- Raisbeck MF, et al. 1981. Lincomycin-associated colitis in horses. *J Am Vet Med Assoc* 179:362.
- Scott DW, et al. 1998. Efficacy of clindamycin hydrochloride capsules for the treatment of deep pyoderma due to *Staphylococcus intermedius* infection in dogs. *Can Vet J* 39:753.
- Sriskandan S, et al. 1997. Comparative effects of clindamycin and ampicillin on superantigenic activity of *Streptococcus pyogenes*. *J Antimicrob Chemother* 40:275.
- Thilsted JP, et al. 1981. Fatal diarrhea in rabbits resulting from the feeding of antibiotic-contaminated feed. *J Am Vet Med Assoc* 179:360.
- Venning CM, et al. 1990. Treatment of virulent foot rot with lincomycin and spectinomycin. *Aust Vet J* 67:258.
- Wulansari R, et al. 2003. Clindamycin in the treatment of *Babesia gibsoni* infections in dogs. *J Am Anim Hosp Assoc* 39:558.

Pleuromutilins: Tiamulin and Valnemulin

Tiamulin and valnemulin are semisynthetic derivatives of the naturally occurring diterpene antibiotic pleuromutilin. Pleuromutilins are mainly active against Gram-positive bacteria with moderate activity against some fastidious Gram-negative bacilli (e.g., anaerobic bacteria) and *Mycoplasma*. The pleuromutilins are used almost exclusively in animals, largely in swine.

Mechanism of action

Pleuromutilin antibiotic derivatives inhibit protein synthesis by binding to the 50S subunit of the bacteria. Tiamulin and valnemulin are strong inhibitors of peptidyl transferase. They can bind concurrently with the macrolide erythromycin but compete with the macrolide carbomycin for binding to the ribosome (Poulsen et al., 2001).

Antimicrobial Activity

Tiamulin and valnemulin have outstanding activity against anaerobic bacteria and *Mycoplasma* (Table 11.1). They are active against some Gram-positive aerobic bacteria including *Staphylococcus* spp., *A. pyogenes*, and some streptococci. Tiamulin is active against only a few Gram-negative aerobic species and

inactive against Enterobacteriaceae (Table 11.1) although subinhibitory concentrations may reduce adhesive properties of enterotoxigenic *E. coli* (Larsen, 1988). Activity against anaerobic bacteria and *Mycoplasma* is better than that of macrolide antibiotics. Organisms with MIC ≤ 4 $\mu\text{g/ml}$ are considered susceptible, of 8–16 $\mu\text{g/ml}$ as moderately susceptible, and ≥ 32 $\mu\text{g/ml}$ as resistant to tiamulin (Szancer, 1990). Valnemulin is about twice as active as tiamulin against bacteria and over 30 times more active against swine mycoplasma in vitro (Aitken et al., 1999).

Resistance

As with the macrolides, resistance to the pleuromutilins occurs from chromosomal mutation. This resistance emerges relatively slowly and in a step-wise fashion on in vitro exposure of bacteria to the drug (Bryskier, 2005). The rate of emergence is significantly lower than with tylosin. There is one-way cross-resistance with tylosin: *Mycoplasma* resistant to tylosin have slightly increased resistance to tiamulin, but *Mycoplasma* resistant to tiamulin are completely resistant to tylosin. There is strain variation in bacterial cross-resistance with the other macrolides and lincosamide antibiotics, which may include modest increases in resistance to spectinomycin and chloramphenicol. A significant increase in the MIC of tiamulin and valnemulin against *Brachyspira hyodysenteriae* isolates has been documented over a 5-year period during which these drugs were used extensively (Lobova et al., 2004). Mutations at the peptidyl transferase center are associated with reduced susceptibility to tiamuline in *Brachyspira* spp. isolates (Pringle et al., 2004).

Pharmacokinetic properties

Tiamulin is used as the hydrogen fumarate in the oral preparation but as the tiamulin base in the parenteral product. Valnemulin is available as a hydrochloride premix for medicated feed. Little information has been published on the pharmacokinetic characteristics of pleuromutilins. In pre-ruminant calves, Ziv et al. (1983) found tiamulin to be rapidly absorbed after oral administration and to have a half-life of 25 minutes following parenteral administration. Tiamulin is a lipophilic, weak organic base, with a pK_a of 7.6. The drug penetrates cells well and is concentrated several fold in milk. Concentration in other tissues is several times that in serum. The half-life in dogs after IM administration was 4.7 hours, and serum concentrations

were higher and maintained for longer when the drug was given by SC injection (Laber, 1988). Tiamulin is almost completely absorbed after oral administration in monogastric species but would be expected to be inactivated by rumen flora if administered orally to ruminants. Administration of tiamulin to swine in medicated feed, rather than by direct oral administration, strongly decreases its rate and extent of absorption and consequently serum concentrations. The bioavailability of valnemulin in pigs exceeds 90% when administered in feed. Similar to what has been described for tiamulin, valnemulin concentrations in the colonic content and tissues exceed serum concentrations. Dosage recommendations are presented in Table 11.2.

Drug interactions

Drug interactions have not been studied extensively but are likely to be similar to those described for lincosamides and macrolides. Tiamulin and valnemulin have been shown to interact with ionophores such as monensin, salinomycin, lasalocid, and narasin. Animals should not receive these products for at least 5 days before or after treatment with pleuromutilins. Severe growth depression, ataxia, paralysis, or death may result (Miller, 1986).

Toxicity and adverse effects

Tiamulin should not be fed at therapeutic concentrations with ionophores such as monensin, narasin, and salinomycin to animals (pigs, poultry) because of the dose-dependent fatal effects of such combinations, which results from tiamulin's potent inducer-inhibiting activity against cytochrome P-450 in the liver.

Intramuscular injection of certain preparations may be irritating, but formulations of tiamulin base in sesame oil are not. Intravenous injection of calves resulted in severe neurotoxicity and death (Ziv et al., 1983). Orally administered tiamulin is transiently unpalatable and irritating in calves.

Acute dermatitis with cutaneous erythema and intense pruritus has been described in pigs following oral administration of tiamulin (Laperle, 1990), where it was associated with poor hygiene and overcrowding. It was suggested that metabolites of tiamulin in urine irritated the skin.

Medication of pigs with valnemulin in the European Union has resulted in adverse effects character-

ized by inappetence, pyrexia, ataxia, and sometimes recumbency. The majority of cases occurred in Denmark and Sweden. In these countries, the incidence of these adverse effects ranged from 0.03 to 1.8% of all pigs treated. On some farms, up to one third of treated pigs were affected, with a mortality rate of 1%. An epidemiological study has suggested an association between susceptibility to these adverse reactions and the Swedish and Danish Landrace breed.

Pleuromutilins should not be administered to horses because of the potential danger for disruption of the colonic microflora and predisposition to enterocolitis.

Clinical applications

Tiamulin or valnemulin are used extensively in swine against *Mycoplasma* pneumonia and swine dysentery. Less commonly, tiamulin has been used against leptospirosis, and to a lesser extent against bacterial pneumonia. Its potential use in bovine respiratory disease appears good. Because of its excellent activity against some bacteria and *Mycoplasma*, tiamulin is preferred over macrolides for many infections.

Cattle, Sheep, and Goats

There are few reports of the use of tiamulin or valnemulin in cattle. Tiamulin has been used successfully to prevent *Mycoplasma bovis* fibrinous polyarthritides and synovitis in veal calves after administration in milk at 400 ppm for the fattening period (Keller et al., 1980). In sheep, tiamulin had a beneficial effect on the course of field cases of infectious rickettsial keratoconjunctivitis (Konig, 1983). Ball and McCaughey (1986) found that a single SC injection of aqueous tiamulin eliminated *Ureaplasma* from the genital tract of 18 of 22 sheep.

Valnemulin administered orally was effective in the control of *Mycoplasma bovis* infections in calves under both experimental and field conditions. In one study, Valnemulin resulted in a more rapid reduction of clinical scores and eliminated *M. bovis* from the lungs more effectively than enrofloxacin (Stipkovits et al., 2005). Valnemulin topical spray has a similar efficacy as lincomycin in the treatment of digital dermatitis in cattle (Laven and Hunt, 2001).

Swine

Tiamulin is used both as a growth promoter in swine and for its outstanding effectiveness against swine

dysentery and chronic pneumonia in swine. It has good activity against *E. rhusiopathiae*, *Leptospira*, and streptococci and moderate activity against *A. pleuropneumoniae*. Tiamulin is used in strategic medication in pig production to prevent and treat common infections. Its activity in vitro against *M. hyopneumoniae* requires confirmation in vivo.

The drug is highly effective in preventing and treating swine dysentery. Concentrations of 60 ppm in water for 3–5 days apparently eradicated experimental infections; relapses occurred when lower concentrations were used (Taylor, 1980). Tiamulin at 30 ppm in feed has prevented dysentery. Incorporation into water (45 ppm for 5 days, 60 ppm for 3 days) effectively treated swine dysentery and was better than tylosin (Pickles, 1982). A single IM dose of 10–15 mg/kg has successfully treated clinical cases of dysentery (Burch et al., 1983). It is approved in the United States for the prophylaxis of swine dysentery. Tiamulin may be used to eradicate swine dysentery from herds using a variety of approaches. These have included daily injection of carrier animals with 10 mg/kg IM for 5 consecutive days, combined with management changes and rodent control (Blaha et al., 1987), or oral administration to grower pigs for 10 days followed by carbadox for 42 days (Moore, 1990).

Tiamulin has had good results in treating field cases of enzootic pneumonia and other *Mycoplasma* infections. In one study, treatment with 200 ppm in the feed for 10 days at weaning significantly reduced lung lesions (Martineau et al., 1980). Administration in the drinking water at 3 mg/kg to pigs with enzootic pneumonia markedly improved average daily weight gain and feed efficiency (Pickles, 1980). In another study, tiamulin was as effective as tulathromycin and florfenicol for reducing fever and attenuating clinical signs during natural outbreaks of respiratory disease in swine. The most common pathogens isolated from affected pigs were *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, and *Mycoplasma hyopneumoniae* (Najjani et al., 2005). Tiamulin has proved superior to tylosin in treating experimental *Mycoplasma* and bacterial pneumonia in swine (Hannan et al., 1982). It has been used successfully in the early medicated weaning procedure of Alexander et al. (1980) to obtain piglets free of endemic pathogens (Meszaros et al., 1985). On the other hand, orally administered tiamulin had no effect in treating swine in the early stages of experimentally induced *M. hyopneumoniae*

pneumonia (Ross and Cox, 1988). This unexpected discrepancy may be explained by the activity of tiamulin against some of the other bacteria and *Mycoplasma* involved in chronic pneumonia of swine but does not explain the poor activity against *M. hyopneumoniae*. Further studies are required on the use of tiamulin in controlling enzootic pneumonia in pigs. Tiamulin has been used with apparent success in eradicating *A. pleuropneumoniae* infection from herds (Larsen et al., 1990) and also in reducing lesions in pigs treated for chronic *A. pleuropneumoniae* infection (Anderson and Williams, 1990).

Tiamulin fed at 200 ppm in feed for 10 days cured chronic kidney carriage of experimental *L. pomona* infection (Laber and Walzl, 1979). Tiamulin administered in drinking water significantly reduced the effects of experimentally-induced *Streptococcus suis* type 2 infection (Chengappa et al., 1990). Tiamulin is effective in the prevention and treatment of proliferative enteropathy (McOrist et al., 1996).

Valnemulin is approved in the European Union for the treatment and prevention of swine enzootic pneumonia, swine dysentery, and proliferative ileitis in pigs. Valnemulin has been shown to be effective for the treatment or prevention of both experimentally induced and naturally acquired infection with *M. hyopneumoniae*, *B. hyodysenteriae*, *B. pilosicoli*, and *L. intracellularis* (Burch 2004). Although valnemulin significantly reduces lung lesions in cases of enzootic pneumonia, *M. hyopneumoniae* is not completely eliminated.

Poultry

Valnemulin and tiamulin in the drinking water have been shown to be effective in the control of *Mycoplasma gallisepticum* infections (Jordan, 1998). Tiamulin was also effective for the treatment of *B. pilosicoli* infections (Stephens and Hampson, 2002).

Bibliography

- Aitken AA, et al. 1999. Comparative in vitro activity of valnemulin against porcine bacterial pathogens. *Vet Rec* 144:128.
- Alexander TJL, et al. 1980. Medicated early weaning to obtain pigs free of pathogens endemic in the herd of origin. *Vet Rec* 106:114.
- Anderson MD, Williams JA. 1990. Effects of tiamulin base administered intramuscularly to pigs for treatment of pneumonia associated with *Actinobacillus (Haemophilus) pleuropneumoniae*. *Int Pig Vet Soc* 15.
- Ball HJ, McCaughey WJ. 1986. Use of tiamulin in the elimination of ureaplasmas from sheep. *Br Vet J* 142:257.
- Blaha T, et al. 1987. Swine dysentery control in the German Democratic Republic and the suitability of injections of tiamulin for the programme. *Vet Rec* 121:416.
- Bryskier A. 2005. Mutilins. In: Bryskier A (ed). *Antimicrobial Agents: Antibacterials and Antifungals*. Washington: ASM Press. 1239-1241.
- Burch DGS, Goodwin RFW. 1984. Use of tiamulin in a herd of pigs seriously affected with *Mycoplasma hyosynoviae* arthritis. *Vet Rec* 115:594.
- Burch DGS, et al. 1983. Tiamulin injection for the treatment of swine dysentery. *Vet Rec* 113:236.
- Burch DGS. 2004. The comparative efficacy of antimicrobials for the prevention and treatment of enzootic pneumonia and some of their pharmacokinetic/pharmacodynamic relationships. *The Pig Journal* 53:8.
- Burch DGS. 2004. Valnemulin for the prevention and treatment of colitis and ileitis. *The Pig Journal* 53:221.
- Chengappa MM, et al. 1990. Efficacy of tiamulin against experimentally induced *Streptococcus suis* type-2 infection in swine. *J Am Vet Med Assoc* 197:1467.
- Hannan PCT, et al. 1982. Tylosin tartrate and tiamulin effects on experimental piglet pneumonia induced with pneumonic pig lung homogenates containing mycoplasma, bacteria, and viruses. *Res Vet Sci* 33:76.
- Jordan FT, et al. 1998. In vitro and in vivo comparisons of valnemulin, tiamulin, tylosin, enrofloxacin, and lincomycin/spectinomycin against *Mycoplasma gallisepticum*. *Avian Dis*. 42:738
- Keller H, et al. 1980. Über Spontan- und Experimenteltalle von Polyarthritis und-synovitis bei Kalbern, verursacht durch Mycoplasmen: (1) Klinische Aspekte. *Schweiz Arch Tierheilkd* 122:15.
- König CDW. 1983. "Pink eye" or "zere oogies" or keratoconjunctivitis infectiosa ovis (k10). Clinical efficacy of a number of antimicrobial therapies. *Vet Quart* 5:122.
- Laber G. 1988. Investigation of pharmacokinetic parameters of tiamulin after intramuscular and subcutaneous administration in dogs. *J Vet Pharm Ther* 11:45.
- Laber G, Walzl H. 1979. Orientierende Prüfung der chemotherapeutischen Wirksamkeit von Tiamulin gegenüber einer experimentellen *Leptospira pomona* Infektion am Schwein. *Wien Tierärztl Monatsh* 66:85.
- Laperle A. 1990. Dermatite aigue chez des porcs traités à la tiamuline. *Med Vet Quebec*:20.
- Larsen H, et al. 1990. Eradication of *Actinobacillus pl europneumoniae* from a breeding herd. *Int Pig Vet Soc*:18.
- Larsen JL. 1988. Effect of subinhibitory concentrations of tiamulin on the haemagglutinating properties of fimbriated *Escherichia coli*. *Res Vet Sci* 45:134.
- Laven RA and Hunt H. 2001. Comparison of valnemulin and lincomycin in the treatment of digital dermatitis by individually applied topical spray. *Vet Rec*. 149:302.
- Lobova D, et al. 2004. Decreased susceptibility to tiamulin and valnemulin among Czech isolates of *Brachyspira hyodysenteriae*. *J Med Microbiol*. 53:287.

- Martineau GP, et al. 1980. Bronchopneumonie enzootique du porc: Amélioration de l'index pulmonaire après traitement par la tiamuline. *Ann Med Vet* 124:281.
- McOrist S, et al. 1996. Treatment and prevention of porcine proliferative enteropathy with oral tiamulin. *Vet Rec* 139:615.
- Meszaros J, et al. 1985. Eradication of some infectious pig diseases by perinatal tiamulin treatment and early weaning. *Vet Rec* 116:8.
- Miller DJS, et al. 1986. Tiamulin/salinomycin interactions in pigs. *Vet Rec* 118:73.
- Moore C. 1990. Eradicating disease in the grower-finisher section. *Compend Contin Educ Pract Vet* 13:329.
- Murdoch RS. 1975. Treatment of atrophic rhinitis. *Vet Rec* 97:251.
- Najiani IA, et al. 2005. Evaluation of the therapeutic activity of tulathromycin against swine respiratory disease on farms in Europe. *Vet Ther.* 6:203.
- Pickles RW. 1980. Field trials in the UK to evaluate tiamulin as a treatment for enzootic pneumonia. *Proc Int Pig Vet Soc* 306.
- Pickles RW. 1982. Tiamulin water medication in the treatment of swine dysentery under field conditions. *Vet Rec* 110:403.
- Poulsen SM, et al. 2001. The pleuromutilin drugs tiamulin and valnemulin bind to the RNA at the peptidyl transferase centre of the ribosome. *Mol Microbiol* 41:1091.
- Prinble M., et al. 2004. Mutations in ribosomal protein L3 and 23S ribosomal RNA at the peptidyl transferase centre are associated with reduced susceptibility to tiamulin in *Brachyspira* spp. isolates. *Mol Microbiol* 2004 54:1295.
- Riond JL, et al. 1993. Influence of tiamulin concentration in feed on its bioavailability in piglets. *Vet Res* 24:494.
- Ross RF, Cox DF. 1988. Evaluation of tiamulin for treatment of mycoplasmal pneumonia in swine. *J Am Vet Med Assoc* 193:441.
- Stephens SP and Hampson DJ. 2002. Evaluation of tiamulin and lincomycin for the treatment of broiler breeders experimentally infected with the intestinal spirochaete *Brachyspira pilosicoli*. *Avian Pathol.* 31:299.
- Stipkovits L, et al. 2005. The efficacy of valnemulin (Econor) in the control of disease caused by experimental infection of calves with *Mycoplasma bovis*. *Res Vet Sci.* 78:207.
- Szancer J. 1990. Minimal inhibitory concentrations of antimicrobial agents against *Actinobacillus pleuropneumoniae*. *Can J Vet Res* 54:198.
- Tasker JB, et al. 1981. Eradication of swine dysentery from closed pig herds. *Vet Rec* 108:382.
- Taylor DJ. 1980. Tiamulin in the treatment and prophylaxis of experimental swine dysentery. *Vet Rec* 106:114.
- Ziv G, et al. 1983. Clinical pharmacology of tiamulin in ruminants. *J Vet Pharm Ther* 6:23.

Streptogramins

Streptogramins are a group of natural (virginiamycin, pristinamycin) or semisynthetic (quinupristin/

dalfopristin) cyclic peptides. The natural streptogramins are produced as secondary metabolites by *Streptomyces* spp. Streptogramins are unique among antibiotics in that each member of the class consists of at least two structurally unrelated molecules: group A streptogramins (macrolactones) and group B streptogramins (cyclic hexadepsipeptides). Virginiamycin has been developed largely as a growth promoter, but pristinamycin and quinupristin/dalfopristin have been developed for clinical use in human medicine, the former for oral administration and the latter for parenteral use. Only virginiamycin has been studied in veterinary species. New streptogramins with improved in vitro activity are currently being investigated (Eliopoulos et al., 2005).

Mechanism of Action

Streptogramins inhibit bacterial protein synthesis by undergoing strong irreversible binding to the 50S ribosomal subunit. The group A and B streptogramins bind to separate sites on the 50S subunit of the bacterial ribosome. Binding of group A streptogramins to the ribosome induces a conformational change which increases affinity of the ribosome for group B compounds. Group A streptogramins prevent peptide bond formation during the chain elongation step, while group B components cause the release of the incomplete peptide chains from the 50S ribosomal subunit. The group B streptogramins share an overlapping binding site with macrolides and lincosamides on the ribosome even though these antimicrobials are structurally unrelated to each other. Individually, the A and B compounds are bacteriostatic, whereas in combination they are bactericidal. Their synergistic activity tends to reduce the emergence of bacterial resistance to either of the combination.

Resistance

Since group A and B streptogramins are chemically unrelated and have different binding sites, the mechanisms of resistance to these two compounds are different. Resistance may be chromosomal or plasmid-mediated. The first and most common mechanism of resistance to streptogramins B is the acquisition of rRNA methylases encoded in the erythromycin-resistant methylase (*erm*) genes. These enzymes add one or two methyl groups to a single adenine in the 23S rRNA moiety. This gives the host bacteria resistance to macrolides, lincosamides, and streptogramins

B (MLSB). The second and less common mechanism for resistance to streptogramins B is linearization of the hexadepsipeptide ring by specific lyases.

Resistance to class A streptogramins is mediated by two mechanisms. The first mechanism is active efflux due to ABC transporter proteins. These proteins pump the drug out of the cell or the cellular membrane, keeping intracellular concentrations low and allowing the ribosome to function. The second mechanism is inactivation of the drug by acetyltransferases.

Virginiamycin

Virginiamycin is an antibiotic mixture of virginiamycin S (group B) and virginiamycin M (group A), produced as a fermentation product of *Streptomyces virginiae*. The drug is mainly active against Gram-positive aerobic and anaerobic bacteria (such as *Clostridium perfringens*). Most Gram-negative bacteria are resistant: *Histophilus somni*, *Lawsonia intracellularis*, *Leptospira* spp., and *B. hyodysenteriae* are exceptions. *Mycoplasma* spp. are often susceptible.

There is little information on the development and prevalence of resistance to virginiamycin. Studies of *C. perfringens* isolated from turkeys and pigs have not identified resistant isolates. Use of virginiamycin as a feed additive may result in the selection of resistant fecal enterococci with cross-resistance to a related streptogramin antibiotic, quinupristin-dalfopristin (Synercid®), which has been used in human medicine for the treatment of vancomycin-resistant enterococci and other infections (see below). The potential benefits of using virginiamycin to control disease in animals may outweigh the risks of an increase in resistant enterococci (Cox, 2005).

There are few data available on the pharmacokinetic properties of virginiamycin in animals. The drug is not absorbed after oral administration. It is safe if administered orally. Virginiamycin is still used in many countries to promote growth in animals at the level of 5–20 ppm (Chapter 24). The use of virginiamycin for this indication was banned by the European Union in 1999 because of resistance in enterococcal isolates. It is administered to swine at 110 ppm in feed to control swine dysentery, but results have sometimes been poor. The drug does not eradicate infection, and duration of treatment should be several weeks. Virginiamycin (Founderguard) has been used to control cecal fermentation and prevent laminitis in horses fed high concentrate rations. Dietary supplementation with

virginiamycin may also lessen some behavioral problems associated with management of stabled horses and the high intake of grain. The suggested mechanism for the improved behavior due to virginiamycin supplementation is reduced fermentative acidosis in the hindgut (Johnson et al., 1998).

Bibliography

- Cox LA, Jr. 2005. Potential human health benefits of antibiotics used in food animals: a case study of virginiamycin. *Environ Int* 31:549.
- Dutta GN, Devriese LA. 1982. Resistance to macrolide, lincosamide and streptogramin antibiotics and degradation of lincosamide antibiotics in streptococci from bovine mastitis. *J Antimicrob Chemother* 10:403.
- Eliopoulos GM, et al. 2005. In vitro activity of an oral streptogramin antimicrobial, XRP2868, against gram-positive bacteria. *Antimicrob Agents Chemother* 7:3034.
- Johnson KG, et al. 1998. Behavioural changes in stabled horses given nontherapeutic levels of virginiamycin. *Equine Vet J* 30:139.
- Roberts MC. 2004. Resistance to macrolide, lincosamide, streptogramin, ketolide, and oxazolidinone antibiotics. *Mol Biotechnol* 28:47.
- Ronne H, Jensen JEC. 1992. Virginiamycin susceptibility of *Serpulina hyodysenteriae* in vitro and in vivo. *Vet Rec* 131:239.
- Thal LA, Zervos MJ. 1999. Occurrence and epidemiology of resistance to virginiamycin and streptogramins. *J Antimicrob Chemother* 43:171.
- Welton LA, et al. 1998. Antimicrobial resistance in enterococci isolated from turkey flocks fed virginiamycin. *Antimicrob Agents Chemother* 42:705.

Pristinamycin and Quinupristin/dalfopristin

Pristinamycin was isolated from *Streptomyces pristinaspiralis*. Pristinamycin has two components: 30 to 40% is pristinamycin IA (group B) and 60 to 70% is pristinamycin IIA (group A). Pristinamycin has been used as an oral antibiotic for humans in France and other European countries since 1968. It is active against Gram-positive bacteria, especially *Staphylococcus* and *Streptococcus* spp., and a few Gram-negative bacteria such as *Haemophilus*, *Neisseria*, and *Legionella* spp. It is also active against *Mycoplasma* spp.

Quinupristin/dalfopristin consists of a mixture of semisynthetic water-soluble derivatives of pristinamycins IA (quinupristin) and IIA (dalfopristin). Its water solubility allows IV administration, making it the first injectable streptogramin available for clinical

use. The combination has a wide distribution in most tissues. In humans, both components are highly protein-bound and are cleared rapidly from plasma via biliary excretion by hepatic conjugation. Phlebitis at the site of infusion is the most common adverse effect. Reversible arthralgia and myalgia occur in up to 5% of treated patients.

The combination of quinupristin/dalfopristin is bactericidal against many Gram-positive bacteria, with selective activity against some fastidious Gram-negative aerobes and Gram-negative anaerobes. Gram-positive bacteria with acquired resistance to macrolides and lincosamides commonly develop resistance to the streptogramin B rather than to the A component of the combination. These features, as well as the properties of high susceptibility among Gram-positive bacteria, make this combination of considerable interest in human medicine for the treatment of susceptible multi-resistant bacteria. Examples include methicillin-resistant *S. aureus* (MRSA) and penicillin- or erythromycin-resistant pyogenic streptococci. An important feature is the activity of the combination against vancomycin-resistant *Enterococcus faecium*. As discussed in Chapter 3, the use of virginiamycin as a growth promoter in animals has raised consider-

able concern that this practice may interfere with the efficacy of the combination for the treatment of vancomycin-resistant enterococcal infections in people. Quinupristin/dalfopristin is also active in vitro against *Streptococcus pneumoniae*, *Neisseria* spp., *Mycoplasma* spp., *Legionella* spp., *Haemophilus* spp., and *Chlamydia* spp. Among the anaerobes, *Clostridium perfringens* and *C. difficile* are the most susceptible. The combination is also active against many other anaerobes including *Fusobacterium* spp. and peptostreptococci.

Bibliography

- Brown J and Freeman BB. 2004. Combining quinupristin/dalfopristin with other agents for resistant infections. *Ann Pharmacother* 38:677.
- Finch RG. 1996. Antibacterial activity of quinupristin/dalfopristin. *Drugs* 1996 Suppl 1:31.
- Pechere J-C. 1996. Streptogramins. A unique class of antibiotics. *Drugs* 51 Suppl 1:13.
- Van den Bogaard AE, et al. 1997. High prevalence of colonization with vancomycin- and pristinamycin-resistant enterococci in healthy humans and pigs in the Netherlands: Is the addition of antibiotics to animal feeds to blame? *J Antimicrob Chemother* 40:454.

Macrolides, Azalides, and Ketolides

Steeve Giguère

Macrolides (macro meaning large and olide meaning lactone) are characterized by having a central 12- to 16-member lactone ring that has few or no double bonds and no nitrogen atoms to which two or more sugar moieties are attached. The efficacy of this group of drugs against important human pathogens, including *Campylobacter*, *Chlamydia*, *Legionella*, and *Mycobacterium* species, has resulted in development of semisynthetic members with increased antibacterial activity, improved pharmacokinetic parameters, and reduced adverse reactions.

The macrolides are classified according to the number of atoms comprising the lactone ring (Figure 12.1). The 12-member ring macrolides are no longer used in clinical practice. Tulathromycin, a semisynthetic macrolide recently approved for use in swine and cattle, consists of a regioisomeric equilibrated mixture of a 13-membered ring (10%) and a 15-membered ring (90%). The unique structural feature of this antimicrobial places it in a novel category of macrolides termed triamilides. The 14-member ring group contains compounds of natural origin (erythromycin and oleandomycin) and semisynthetic derivatives (clarithromycin, roxithromycin, dirithromycin). The 15-member ring is represented by azithromycin and one isomer of tulathromycin. The 15-membered ring macrolides are named azalides, as they have a nitrogen atom in the lactone ring. The 16-member group also contains both compounds of natural origin (spiramycin, josamycin, midecamycin) and semisynthetic derivatives (rokitamycin, miokamycin).

The use of macrolides in animals has been somewhat limited due to toxicities associated with oral administration to herbivores and pain associated with IM administration. As a class, the macrolides exhibit high intracellular concentrations, broad distribution

in tissue and, in the case of new drugs, prolonged half-lives. They also exhibit excellent activity against many important bacterial pathogens of animals. Despite all of these advantages they are, for the most part, underutilized in veterinary medicine. The macrolides are also known for their intracellular accumulation within phagocytes. This may enhance the host's immunomodulatory effect (also seen with lincosamides), but the precise pharmacodynamic relationships between intracellular concentrations and bacterial killing remain to be defined.

Mechanism of Action

Macrolides inhibit protein synthesis by reversibly binding to 50S subunits of the ribosome. They inhibit the transpeptidation and translocation process, causing premature detachment of incomplete polypeptide chains. Their binding sites on the 23S rRNA of the 50S ribosomal subunit overlap with that of clindamycin but are different from those of chloramphenicol. Macrolides are generally bacteriostatic agents. They may be bactericidal at high concentrations and against a low inoculum of highly susceptible bacteria.

Resistance

Three different mechanisms account for bacterial resistance to the action of macrolides: (1) target site modification; (2) active efflux; and (3) enzymatic inactivation. Target site modification and active efflux are responsible for the majority of resistant isolates.

Target site modification has resulted in cross-resistance to the macrolides, lincosamides and streptogramin B (MSLB resistance). Different bacterial species are able to synthesize an enzyme encoded by a series of structurally related erythromycin-resistant methylase (*erm*) genes that methylates RNA. These

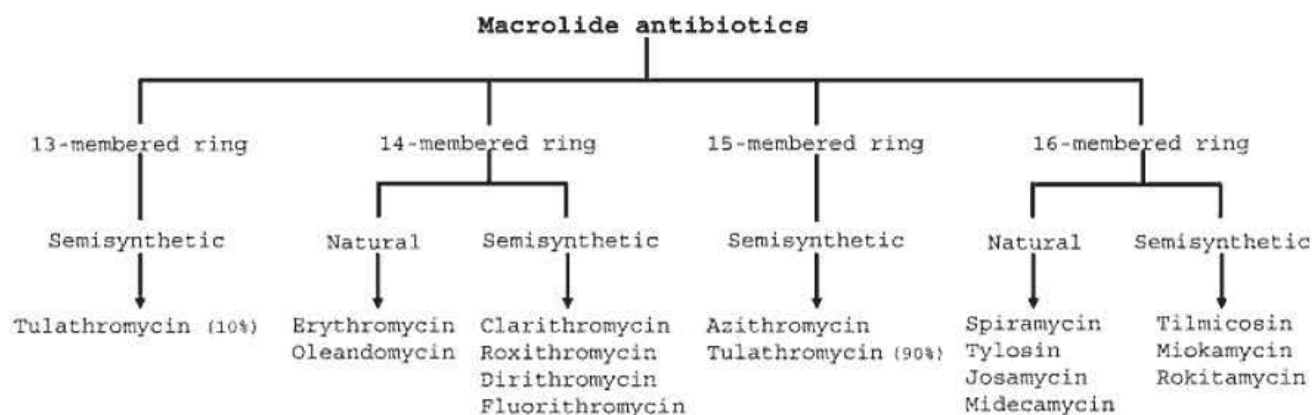


Figure 12.1. Classification of macrolide antimicrobials according to the size of the macrocyclic lactone ring.

methyltransferase genes are widely distributed in both Gram-positive and Gram-negative bacteria and can be located on plasmids and transposons. The *erm* genes can be expressed constitutively or inducibly. Constitutive resistance occurs when the methylase enzyme is inherently produced. Inducible resistance occurs when enzyme induction is effected by exposure of the microorganism to 14-member or 15-member, but not 16-member, ring macrolides.

The second mechanism of resistance, active efflux, is mediated by the *mef* gene. This leads to the M phenotype which is resistance to 14- and 15-member ring macrolides and susceptibility to 16-member ring macrolides, ketolides, lincosamides and streptogramin B. The *mef* genes have been found in a variety of Gram-positive bacteria. The last mechanism of resistance is due to enzymatic degradation of the lactone ring. The clinical significance of this last mechanism has not been clearly established.

Drug Interactions

There have been few studies of the interactions of macrolide antibiotics with other antimicrobial drugs. Combinations of erythromycin with other macrolides, lincosamides, and chloramphenicol are antagonistic in vitro. Erythromycin has been used alone or with an aminoglycoside to prevent or treat peritonitis after intestinal spillage, but it is not as effective as clindamycin or metronidazole in combination with an aminoglycoside. Combination of a macrolide and a fluoroquinolone or aminoglycoside may be synergistic, antagonistic, or indifferent, depending on the microorganism studied. Combination of a macrolide with

rifampin gave synergistic inhibition of *Rhodococcus equi* (Prescott and Nicholson, 1984).

Erythromycin and many other macrolides lead to inactivation of the cytochrome P_{450} enzyme complex. Concurrent administration of erythromycin increases concentrations of drugs that are primarily dependent upon CYP3A metabolism such as theophylline, midazolam, carbamazepine, omeprazole and ranitidine. Clarithromycin and roxithromycin have lower affinity for the P_{450} system than erythromycin and other classic macrolides (except spiramycin). Azithromycin, dirithromycin and spiramycin do not interact with the hepatic cytochrome P_{450} system and are not associated with the drug interactions observed with erythromycin and other macrolides.

Anti-inflammatory and Prokinetic Activities of Macrolides

Macrolides have immunomodulatory effects that are beneficial for humans suffering from many inflammatory pulmonary diseases. These effects are independent of the antibacterial activity of these drugs. Erythromycin, azithromycin, clarithromycin, and roxithromycin inhibit chemotaxis and infiltration of neutrophils into the airway and, subsequently, decrease mucus secretion. The mechanisms of action for the anti-inflammatory properties of the macrolides are multifactorial and still being investigated (Tamaoki et al., 2004). Macrolides inhibit the production of many proinflammatory cytokines including interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor- α , perhaps by suppressing the transcription factor nuclear factor- κ B or activator protein-1.

Inhibition of cytokine production has been seen in vitro and also in bronchoalveolar lavage fluid. Macrolides also inhibit formation of leukotriene B₄, which attracts neutrophils, and inhibit the release of superoxide anion by neutrophils that may be present in the airway. Macrolides also block formation of adhesion molecules necessary for neutrophil migration. These anti-inflammatory effects have been described in foals receiving erythromycin (Lakritz et al., 1997), and in cattle and pigs administered tilmicosin (Lakritz et al., 2002; Nerland et al., 2005).

Macrolides with a 14-member ring and a glycosidic linkage of the lactone ring such as erythromycin and clarithromycin have prokinetic effects on the gastrointestinal tract by acting as motilin receptor agonists. These effects have been demonstrated in horses (Lester et al, 1998), cattle (Wittek and Constable, 2005), and dogs (Cowles et al., 2000).

Bibliography

- Cowles VE, et al. 2000. Effect of novel motilide ABT-229 versus erythromycin and cisapride on gastric emptying in dogs. *J Pharmacol Exp Ther.* 293:1106.
- Lakritz J, et al. 1997. Effect of treatment with erythromycin on bronchoalveolar lavage fluid cell populations in foals. *Am J Vet Res.* 58:56.
- Lakritz J, et al. 2002. Tilmicosin reduces lipopolysaccharide-stimulated bovine alveolar macrophage prostaglandin E(2) production via a mechanism involving phospholipases. *Vet Ther.* 3:7.
- Lester GD, et al. 1998. Effect of erythromycin lactobionate on myoelectric activity of ileum, cecum, and right ventral colon, and cecal emptying of radiolabeled markers in clinically normal ponies. *Am J Vet Res.* 59:328.
- Nerland EM, et al. 2005. Effects of oral administration of tilmicosin on pulmonary inflammation in piglets experimentally infected with *Actinobacillus pleuropneumoniae*. *Am J Vet Res.* 66:100.
- Tamaoki J, et al. 2004. Clinical implications of the immunomodulatory effects of macrolides. *Am J Med.* 117 Suppl 9A:5S.
- Wittek T, Constable PD. 2005. Assessment of the effects of erythromycin, neostigmine, and metoclopramide on abomasal motility and emptying rate in calves. *Am J Vet Res.* 66:545.

Macrolides Approved for Veterinary use: Erythromycin, Tylosin, Spiramycin, Tilmicosin, Tulathromycin

Erythromycin

Erythromycins are produced as a complex of six components (A to F) by *Saccharopolyspora erythraea* (formerly *Streptomyces erythraeus*). Only erythromycin A has been developed for clinical use. Erythromycin has a macrocyclic lactone nucleus to which ketones and amino sugars are attached (Figure 12.2). Its base has a pK_a of 8.8, it is poorly soluble in water, and is unstable in gastric acid.

Antimicrobial Activity

Good susceptibility (MIC ≤ 0.5 µg/ml) is shown in the following Gram-positive aerobes: *Arcanobacterium pyogenes*, *Bacillus* spp., *Corynebacterium* spp., *Rhodococcus equi*, *Erysipelothrix rhusiopathiae*, *Listeria* spp.,

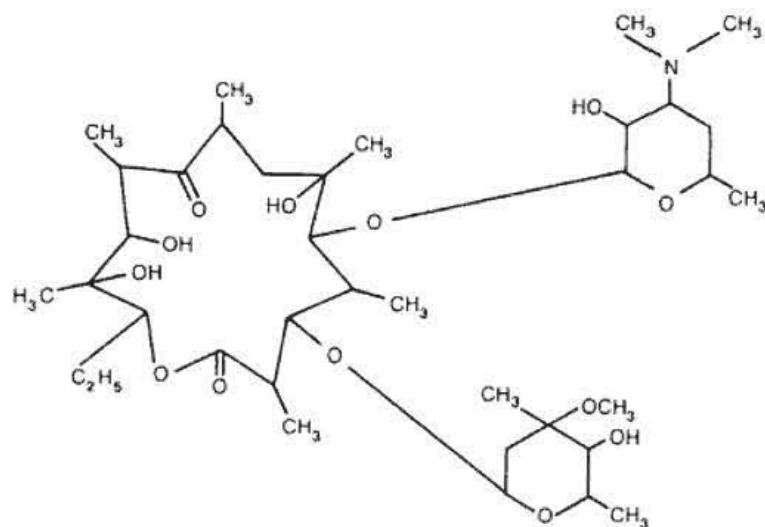


Figure 12.2. Structural formula of erythromycin.

staphylococci, streptococci. Among Gram-negative aerobes: *Actinobacillus* spp., *Brucella* spp.; *Campylobacter* spp., *Leptospira* spp. Anaerobic bacteria: *Actinomyces* spp., *Bacteroides* spp. (except *B. fragilis*), *Clostridium* spp., some *Fusobacterium* spp., and anaerobic cocci. Erythromycin is also active against some *Chlamydia/Clamydophila* spp. and *Mycoplasma* spp. (Table 12.1).

Moderate susceptibility (MIC 1–4 µg/ml) occurs in enterococci, some *Bordetella* spp., *Haemophilus* spp., *Legionella* spp., *Ehrlichia* spp., *Pasteurella* spp.

Resistant (MIC \geq 8 µg/ml) bacteria include all Enterobacteriaceae, *Pseudomonas* spp., *Nocardia* spp., *Mycobacterium* spp. (other than *M. kansasii*), and some *Mycoplasma* spp.

Pharmacokinetic Properties

The erythromycin base is highly susceptible to degradation from gastric acids. To circumvent this, orally administered erythromycin requires an enteric coating. However, this leads to considerable individual variation in absorption. Erythromycin is available for oral administration as the free base, the stearate or phosphate salts, and as estolate or ethylsuccinate esters. The stearate is hydrolyzed in the intestine to the base, and the ethylsuccinate and estolate esters are absorbed as such and hydrolyzed in the body to the active base. Feeding interferes quite markedly with oral absorption. Like all macrolides, erythromycin is well distributed in the body, being concentrated in tissues, although penetration in cerebrospinal fluid is low. Prostatic fluid concentrations are approximately half that of serum concentration. The drug is metabolised and excreted largely in the bile and, although some intestinal reabsorption occurs, most is lost in feces. Urinary excretion is only 3–5% of the total administered dose.

Erythromycin is available for parenteral injection as the base, glucoheptonate, or lactobionate. Parenteral administration causes tissue irritation at the site of administration.

Toxicity and Adverse Effects

The incidence of serious adverse effects is relatively low and the incidence depends on the animal species. One problem shared with all macrolides is their irritating nature which leads to severe pain on IM injection, thrombophlebitis and periphlebitis after IV

injection, and an inflammatory reaction after intramammary administration. Dose-related gastrointestinal disturbances (nausea, vomiting, diarrhea, intestinal pain) occur in many animals treated with erythromycin, either as a result of disruption of the normal intestinal microflora, or as a result of stimulatory effects on smooth muscle because erythromycin binds motilin receptors. These are not life threatening except in adult horses, where macrolides, because they are largely excreted in the bile, can lead to serious diarrheic illness. Deaths have occurred due to *Clostridium difficile* in adult horses administered erythromycin (Gustafsson et al., 1997). Interestingly, severe *C. difficile* diarrheal illness has also developed in the mares of foals treated orally with erythromycin and rifampin for *Rhodococcus equi* infection. This may be a direct effect of mares ingesting small quantities of antibiotic from the feces of their foals or an indirect effect of mares acquiring erythromycin-resistant *C. difficile* infection from their foals, or a combination of these circumstances (Baverud et al., 1998). Deaths from typhlocolitis have also been reported in rabbits. Oral administration of erythromycin has caused severe diarrhea in ruminating calves. Because of these effects and poor absorption, oral administration of erythromycin to cattle is not recommended. The drug appears safe in dogs and cats. The estolate form has been associated with self-limiting cholestatic hepatitis and jaundice with abdominal pain, especially with repeated and prolonged use or in patients with preexisting hepatic disease.

Other adverse effects of erythromycin in foals include hyperthermia and respiratory distress, which may be more marked in foals kept under high environmental temperatures (Traub-Dargatz et al., 1996).

Administration and Dosage

Dosages of erythromycin are shown in Table 12.2. When administered IV, erythromycin must be diluted and administered by slow infusion to prevent adverse reactions.

Clinical Applications

Erythromycin is a drug of choice to prevent or treat *Campylobacter jejuni* diarrhea or abortion. Erythromycin is an alternative to penicillin in penicillin-allergic animals in the treatment of infections caused by susceptible Gram-positive aerobes, a less useful alternative to clindamycin or metronidazole in anaero-

bic infections, an alternative to ampicillin or amoxicillin in the treatment of leptospirosis, and an alternative to tetracyclines in rickettsial infections. The generally bacteriostatic nature of the drug is a disadvantage of this and other macrolides.

Cattle, Sheep, and Goats. Erythromycin has limited use in respiratory disease, as *H. somni*, *A. pyogenes*, and anaerobic bacteria are often moderately susceptible, and some mycoplasma and most *Mannheimia haemolytica* isolates are resistant. Because of extreme pain associated with parenteral injection, it should be avoided when other antimicrobial drugs are available. This antibiotic is perhaps most useful in its intramammary infusion form for lactating and dry-cow therapy of mastitis where it has a short withdrawal time (36 hours). A single IM injection of 10 mg/kg was effective in the treatment of virulent footrot in sheep (Ware et al., 1994).

Swine. Erythromycin has little place in the treatment of swine infections. Exceptions may include leptospirosis (Alt and Bolin, 1996) and *Lawsonia intracellularis* infection (McOrist et al., 1995).

Horses. Erythromycin is an alternative to penicillin G or trimethoprim-sulfonamide in the treatment of staphylococcal and streptococcal infections. The potential for inducing diarrhea limits its use in adult horses. Erythromycin is a drug of choice in the treatment of *Rhodococcus equi* pneumonia in foals and should be used in combinations with rifampin, both for the synergistic effect and to prevent the emergence of resistant mutants. Intramuscular injection causes severe local irritation in horses. The combination of orally administered erythromycin and rifampin successfully treated experimentally induced *Neorickettsia risticii* infection and may represent an alternative to tetracyclines (Palmer and Benson, 1992). Erythromycin, alone or in combination with rifampin, is also the treatment of choice for *Lawsonia intracellularis* infections in foals (Lavoie et al., 2000).

Dogs and Cats. Erythromycin may be a second choice for infections caused by Gram-positive cocci and anaerobic bacteria. It is the drug of choice in treating *C. jejuni* enteritis (Monfort et al., 1990).

Poultry. Erythromycin is administered in water for the prevention and treatment of staphylococcal or

streptococcal infection, necrotic dermatitis, infectious coryza, and *M. gallisepticum* infection.

Bibliography

- Alt DP, Bolin CA. 1996. Preliminary evaluation of antimicrobial agents for treatment of *Leptospira interrogans* serovar pomona infection in hamsters and swine. *Am J Vet Res* 57:59.
- Baverud V, et al. 1998. *Clostridium difficile* associated with acute colitis in mares when their foals are treated with erythromycin and rifampicin for *Rhodococcus equi* pneumonia. *Equine Vet J* 30:482.
- Gustafsson A, et al. 1997. The association of erythromycin ethylsuccinate with acute colitis in horses in Sweden. *Equine Vet J* 29:314.
- Lavoie JP, et al. 2000. Equine proliferative enteropathy: a cause of weight loss, colic, diarrhoea and hypoproteinaemia in foals on three breeding farms in Canada. *Equine Vet J* 32:418.
- McOrist S, et al. 1995. Antimicrobial susceptibility of ileal symbiont intracellularis isolated from pigs with proliferative enteropathy. *J Clin Microbiol* 33:1314.
- Monfort JD, et al. 1990. Efficacies of erythromycin and chloramphenicol in extinguishing fecal shedding of *Campylobacter jejuni* in dogs. *J Am Vet Med Assoc* 196:1069.
- Palmer JE, Benson CE. 1992. Effect of treatment with erythromycin and rifampin during the acute stages of experimentally induced equine ehrlichial colitis in ponies. *Am J Vet Res* 53:2071.
- Traub-Dargatz J, et al. 1996. Hyperthermia in foals treated with erythromycin alone or in combination with rifampin for respiratory disease during hot environmental conditions. *Am Assoc Equine Pract* 42:243.
- Ware JKW, et al. 1994. Efficacy of erythromycin compared with penicillin/streptomycin for the treatment of virulent footrot in sheep. *Aust Vet J* 71:89.

Tylosin

Tylosin is a macrolide antibiotic isolated from *Streptomyces fradiae*. Its chemical structure and mechanism of action are similar to other macrolide antibiotics.

Antimicrobial Activity

Tylosin has a similar spectrum of activity to erythromycin. It is less active against bacteria, except for *B. hyodysenteriae*, but more active against a broad range of *Mycoplasma* spp. (Table 12.1).

Pharmacokinetic Properties

The pharmacokinetic properties of tylosin are characteristic of the macrolides in general. Tylosin is a weak base (pK_a 7.1) and is highly lipid soluble. The elimina-

tion half-life in dogs and cattle is about 1 hour, with apparent volumes of distribution of 1.7 and 1.1 L/kg, respectively. The half-life is considerably longer in sheep, goats and pigs, approximately 4 hours.

Toxicity and Adverse Effects

Tylosin is a relatively safe drug. Its toxic effects are generally similar to those reported for erythromycin. It was reported to cause fatal diarrhea in a horse. The drug is irritating to tissue when administered IM or SC. Pigs have been reported to react to injection by developing edema, pruritus, edema of rectal mucosa, and mild anal protrusion. These effects may be attributed to the drug vehicle. Inadvertent feeding of dairy cows with a concentrate contaminated with 7–20 ppm of tylosin resulted in ruminal stasis, inappetence, foul-smelling feces, and decreased milk production. Many of the cows became hyperesthetic and some became recumbent (Crossman and Poyser, 1981). Intravenous administration in cattle has produced shock, dyspnea, and depression. Tylosin and spiramycin have induced contact dermatitis in veterinarians.

Administration and Dosage

Tylosin is administered by IM injection (Table 12.2), by the intramammary route, or for feed incorporation in swine. Tylosin tartrate is readily absorbed from the intestine, but tylosin phosphate is relatively poorly absorbed.

Clinical Applications

Tylosin is not as active as erythromycin against most bacteria but has greater activity against *Mycoplasma* spp. In pigs, where it is also used as a growth promoter, its use in the prevention and treatment of swine dysentery and *Mycoplasma* infections is being replaced by the more active tiamulin. Apart from its use against *Mycoplasma* it is, like erythromycin, a second-choice antibiotic for most clinical uses.

Cattle, Sheep, and Goats. Tylosin is used in cattle to treat pneumonia, middle ear infections in calves, foot rot, metritis, pinkeye, and mastitis caused by Gram-positive cocci because of its activity against *Mycoplasma*, anaerobic bacteria, and Gram-positive bacteria. Tylosin may be administered at low concentrations to feedlot cattle on high-concentrate diets to improve weight gain and feed efficiency and to prevent liver ab-

scence. Because of the availability of newer macrolide antibiotics, this is now the major use of tylosin.

In cattle, tylosin (7.5 to 15 mg/kg IM twice a day) has been successful in controlling and eliminating experimental *Mycoplasma mycoides* pneumonia. In calves the drug has been used effectively to treat *Mycoplasma bovis* pneumonia and arthritis. However, in studies where tylosin was dosed IM at 10 mg/kg twice a day it delayed, but did not prevent, experimentally induced *M. bovis* arthritis (Stahlheim, 1976). Tylosin has been used via the intramammary route in the treatment of experimentally induced *Mycoplasma californicum* mastitis (Ball and Campbell, 1989). Treatment was most successful when oxytetracycline and tylosin were combined or alternated 426 g of tylosin or 500 g of oxytetracycline given every 12 hours for three days.

Tylosin can be administered IM or by the intramammary route in the treatment of mastitis caused by Gram-positive bacteria. Results have been marginally less successful than with erythromycin. A dose of 20 mg/kg IM was used to eliminate *Ureaplasma* from the genital tract of ewes (Ball and McCaughey, 1987). In goats, tylosin is a drug of choice in treating *Mycoplasma* pneumonia, such as that caused by *M. mycoides* spp. *capri*. A high dosage of 25–35 mg/kg IV at 8- to 12-hour intervals is recommended.

Swine. Tylosin is used to promote growth and improve weight gain. For the treatment of atrophic rhinitis, injection of piglets for variable periods has reduced frequency of the disease, suggesting that tylosin inhibits *Pasteurella multocida* (or its production of Pmt toxin), despite the bacteria's relatively high MIC. Injection of neonatal pigs has reduced the frequency of *M. hyopneumoniae* lesions (Kunesh, 1981). Tylosin was not as effective as tiamulin in controlling an experimental mixed *Mycoplasma* and bacterial pneumonia (Hannan et al., 1982). Tylosin, 8.8 mg/kg twice a day IM, or tylosin-sulfonamide, 100 ppm of each drug in feed, was effective in treating pigs with experimentally induced *P. multocida* and *A. pyogenes* pneumonia (Matsuoka et al., 1983).

Control of swine dysentery by the drug is hampered by the development of resistance; the in vivo effect of the drug varies with the MIC, which ranges from 4 to >32 µg/ml. Derivatives of tylosin may have greater activity against resistant organisms (Jacks et al., 1986). Tylosin (100 ppm) is effective in preventing or treating

proliferative enteropathy (McOrist et al., 1997). Other uses include parenteral treatment of erysipelas and infections involving *A. pyogenes* and anaerobes. Tylosin (44 mg/kg IM once daily for five days) effectively treated experimentally induced leptospirosis in swine (Alt and Bolin, 1996).

Horses. Injection of tylosin has been fatal to horses. There is no experience with its oral administration but no indication for such use, which might be likely to result in enterocolitis.

Dogs and Cats. Tylosin has been used successfully in dogs to treat abscesses, wound infections, tonsillitis, tracheobronchitis, and pneumonia caused by pathogens such as staphylococci, streptococci, anaerobes, and *Mycoplasma*. Occasional pain and swelling at the injection site and vomiting after oral administration have been reported. A tylosin-sulfonamide combination is licensed for the treatment of upper respiratory tract infections in dogs. Tylosin is often effective in the treatment of the upper respiratory tract infection complex of cats, possibly because of its effect against *Clamydophila* and *Mycoplasma*. Tylosin administered orally has been 70–90% effective in the treatment of *Staphylococcus intermedius* pyoderma in dogs (Scott et al., 1994; Harvey, 1996); a dose of 10 mg/kg q12 hours was shown to be almost as effective as 20 mg/kg q12 hours (Scott et al., 1996). Therapy with oral tylosin has been successful for the attenuation of diarrhea in dogs with chronic enteropathies for which specific causes have been ruled out (Westermarck et al., 2005).

Poultry. Tylosin has been used by IM injection in the control of *Mycoplasma* infections and added to the water in the control of avian spirochetosis. Resistance in some *M. gallisepticum* isolates may reduce the efficacy of tylosin (Migaki et al., 1993). In one study, tylosin was found to be almost as effective as danofloxacin in control of infection caused by *Mycoplasma gallisepticum* in broiler chickens (Jordan et al., 1993).

Bibliography

Aarestrup FM, et al. 1998. Surveillance of antimicrobial resistance in bacteria isolated from food animals to antimicrobial growth promoters and related therapeutic agents in Denmark. *APMIS* 106:606.

- Alt DP, Bolin CA. 1996. Preliminary evaluation of antimicrobial agents for treatment of *Leptospira interrogans* serovar *pomona* infection in hamsters and swine. *Am J Vet Res* 57:59.
- Ball HJ, Campbell JN. 1989. Antibiotic treatment of experimental *Mycoplasma californicum* mastitis. *Vet Rec* 125:377.
- Ball HJ, McCaughey WJ. 1987. Experimental intramuscular inoculation of tylosin in the elimination of ureaplasmas from ewes. *Vet Rec* 120:557.
- Crossman PJ, Poyser MR. 1981. Effect of inadvertently feeding tylosin and tylosin and dimetridazole to dairy cows. *Vet Rec* 108:285.
- Hannan PCT, et al. 1982. Tylosin tartrate and tiamulin effects on experimental piglet pneumonia induced with pneumonic pig lung homogenate containing mycoplasma, bacteria, and viruses. *Res Vet Sci* 33:76.
- Harvey RG. 1996. Tylosin in the treatment of canine superficial pyoderma. *Vet Rec* 139:185.
- Jacks TM, et al. 1986. 3-acetyl-4'-isovaleryl tylosin for prevention of swine dysentery. *Am J Vet Res* 47:2325.
- Johnston WS. 1975. Eradication of *Str. agalactiae* from infected herds using erythromycin. *Vet Rec* 96:430.
- Jordan FTW, et al. 1993. A comparison of the efficacy of danofloxacin and tylosin in the control of *Mycoplasma gallisepticum* infection in broiler chickens. *J Vet Pharm Ther* 16:79.
- Kunesh JP. 1981. A comparison of two antibiotics in treating *Mycoplasma pneumonia* in swine. *Vet Med Small Anim Clin* 76:871.
- Matsuoka T, et al. 1983. Therapeutic effect of injectable tylosin against induced pneumonia in pigs. *Vet Med Small Anim Clin* 78:951.
- McOrist S, et al. 1997. Oral administration of tylosin phosphate for treatment and prevention of proliferative enteropathy in pigs. *Am J Vet Res* 58:136.
- Migaki TT, et al. 1993. Efficacy of danofloxacin and tylosin in the control of mycoplasmosis in chicks infected with tylosin-susceptible or tylosin-resistant field isolates of *Mycoplasma gallisepticum*. *Avian Dis* 37:508.
- Scott DW, et al. 1994. Efficacy of tylosin tablets for the treatment of pyoderma due to *Staphylococcus intermedius* infection in dogs. *Can Vet J* 35:617.
- Scott DW, et al. 1996. Further studies on the efficacy of tylosin tablets for the treatment of pyoderma due to *Staphylococcus intermedius* infection in dogs. *Can Vet J* 37:617.
- Stahlheim OHV. 1976. Failure of antibiotic therapy in calves with mycoplasmal arthritis and pneumonia. *J Am Vet Med Assoc* 189:1096.
- Westermarck E, et al. 2005. Tylosin-responsive chronic diarrhea in dogs. *J Vet Intern Med.* 19:177.

Spiramycin

Spiramycin is several times less active against bacteria than erythromycin. Its spectrum of activity is similar to that of the other macrolides, but it is not as effective against *Mycoplasma* as tylosin or tiamulin. Resistance,

antimicrobial drug interactions, and toxic properties are similar to those of the other macrolides.

Despite relatively poor activity in vitro, spiramycin has quite exceptional ability to concentrate in tissue, in part by tissue binding, resulting in concentrations in organs reaching 25–60 times those of serum. The drug persists even when serum concentrations are negligible. Thus, spiramycin has the paradoxical effect of being less active than erythromycin in vitro but as or more active in vivo. Like other macrolides, it also has a direct effect on phagocytic cells. Thus, spiramycin has particular potential against intracellular organisms. In humans, it is used in the treatment of acute toxoplasmosis and may be useful in cryptosporidiosis (Chang and Pechère, 1988). In calves, Schilferli et al., 1981 found that a parenteral administration of 50 mg/kg twice a day for 5 days resulted in lung concentrations of approximately 100 µg/g. Not all this drug is active; in mammary tissue about 75% is inactive. One result of its tissue concentration is the persistence of drug residues for prolonged periods, a particular problem in the treatment of mastitis in lactating cows but also more generally in food animals. Spiramycin is used extensively in France for the treatment of infections in farm animals. It has the same applications as tylosin.

Spiramycin was used extensively in Europe as a broiler chicken growth promoter prior to the ban on these products by the European Union. Resistance in bacteria isolated from chickens fed spiramycin is extensive in Europe (Aarestrup et al., 1998).

Cattle, Sheep and Goats

Spiramycin (pK_a 8.2) has similar applications to tylosin. The drug has been used successfully to treat contagious bovine pleuropneumoniae when administered at 25 mg/kg IM at 48 hour intervals for 3 doses (Provost, 1974). In one field trial of the treatment of bovine respiratory disease, spiramycin was considerably less effective than florfenicol (Madelenat et al., 1997). In another study, a dose of 20 mg/kg gave mastitic milk concentrations greater than 2.5 µg/ml for 48 hours after IM injection. Intramuscular injection of this dose after the last milking gave effective milk drug concentrations for six to eight days (Ziv, 1974). In lactating cows, a single intramammary dose of 600 mg gave effective concentrations for 36–48 hours, but persistent residues limit the use of the drug. Parenteral administration of spiramycin for three to five days did not give satisfactory results in mastitis caused by

penicillin-resistant *S. aureus* (Pyorala and Pyorala, 1998). Spiramycin administered orally to ewes, 100 mg/kg, in the last third of gestation effectively to prevent experimental *Toxoplasma* abortion. Bioavailability after oral administration is limited in ruminants. Spiramycin, administered at 20–30 mg/kg IM, successfully treated ovine infectious rickettsial keratoconjunctivitis; in serious cases the drug should be repeated 5 and 10 days after the first injection (Konig, 1983). One interesting possible application is the use of a single injection of the parenteral dosage form of the drug to treat endometritis in sheep and cattle, because of the extraordinarily long half-life of the drug (Cester et al., 1990).

Swine and Poultry

Spiramycin has the same applications as tylosin in pigs and poultry.

Bibliography

- Aarestrup FM, et al. 1998. Surveillance of antimicrobial resistance in bacteria isolated from food animals to antimicrobial growth promoters and related therapeutic agents in Denmark. *APMIS* 106:606.
- Cester CC, et al. 1990. Spiramycin concentrations in plasma and genital-tract secretions after intravenous injection in the ewe. *J Vet Pharm Ther* 13:7.
- Chang HR, Pechère J-CF. 1988. Activity of spiramycin against *Toxoplasma gondii* in vitro, in experimental infections and in human infection. *J Antimicrob Chemother* 22B:87.
- Konig CDW. 1983. "Pink eye" or "zere oogjes" or keratoconjunctivitis infectiosa ovis (KIO). Clinical efficacy of a number of antimicrobial therapies. *Vet Q* 5:122.
- Madelenat A, et al. 1997. Efficacité comparée du florfenicol et de la spiramycine longue action, associé à la flunixin meglumine, dans le traitement des maladies respiratoires du veau de boucherie. *Rec Med Vet* 173:113.
- Provost A. 1974. Essai de traitement de la spiramycin chez les brebis et les vaches laitières. *Can Med Vet* 43:140.
- Pyorala SH, Pyorala EO. 1998. Efficacy of parenteral administration of three antimicrobial agents in treatment of clinical mastitis in lactating cows: 487 cases (1989-1995). *J Am Vet Med Assoc*. 212:407.
- Renard L, et al. 1996. Pharmacokinetic-pharmacodynamic model for spiramycin in staphylococcal mastitis. *J Vet Pharm Ther* 19:95.
- Schilferli D, et al. 1981. Distribution tissulaire de la pénicilline, de l'oxytetracycline et de la spiramycine chez le veau au cours d'une antibiotique courante. *Schweiz Arch Tierheilkd* 123:507.
- Ziv G. 1974. Profil pharmacocinétique de la spiramycine chez les brebis et les vaches laitières. *Can Med Vet* 43:371.

Tilmicosin

Tilmicosin, 20-deoxy-20-(3,5-dimethylpiperidin-1-yl) desmycosin, is a semisynthetic derivative of tylosin.

Antimicrobial Activity

Tilmicosin has antibacterial and antimycoplasma activity between that of erythromycin and tylosin (Table 12.1). Typical of macrolides, it inhibits Gram-positive bacteria including *Clostridium* spp., *Staphylococcus* spp., and *Streptococcus* spp., Gram-negative bacteria including *Actinobacillus* spp., *Campylobacter* spp., *Haemophilus* spp., and *Pasteurella* spp. All Enterobacteriaceae are resistant. *Mycoplasma* susceptibility can be quite variable because of resistance. *Mannheimia*/*Pasteurella* spp. isolated from cattle are regarded as susceptible if their MIC is ≤ 8 $\mu\text{g/ml}$, intermediate if MIC is 16 $\mu\text{g/ml}$, and resistant if their MIC is ≥ 32 $\mu\text{g/ml}$ (Shryock et al., 1996).

Pharmacokinetic Properties

The pharmacokinetic properties of tilmicosin are similar to that of macrolides in general, and are characterized by low serum concentrations but large volumes of distribution (>2 L/kg), with accumulation and persistence in tissues including the lung, which may concentrate drug 20-fold compared to serum. The half-life in cows is about one hour. Cows administered 10 mg/kg SC as a single dose maintained milk concentrations >0.8 $\mu\text{g/ml}$ for eight to nine days (Ziv et al., 1995). Tilmicosin is rapidly absorbed and slowly eliminated (elimination half-life of 25 h) after oral administration to pigs (Shen et al., 2005).

Toxicity and Adverse Effects

Tilmicosin is potentially toxic to the cardiovascular system, which varies to some extent with species. The drug is fatal to swine when administered by IM injection at doses ranging 10–20 mg/kg. Care should be taken to avoid accidental injection of people. The toxic dose for goats is only about 30 mg/kg SC, or ≥ 2.5 mg/kg IV. Safety has not been determined in horses. The toxic effects of tilmicosin are mediated through its effects on the heart, possibly through causing rapid depletion of calcium (Main et al., 1996).

Administration and Dosage

Administration is summarized in Table 12.2.

Clinical Applications

Cattle and Sheep, and Goats. Tilmicosin has been developed as a long-acting formulation for use in bovine respiratory disease. A single SC dose of 10 mg/kg results in lung concentrations exceeding the MIC of *M. haemolytica* for 72 hours. Experimental and field data support the value of single-dose SC prophylaxis on arrival of cattle in feedlots and in the treatment in pneumonia of cattle (Ose and Tonkinson, 1988; Schumann et al., 1991; Young, 1995; Musser et al., 1996; Rowan et al., 2004). Doses of 20 mg/kg appeared slightly more effective than 10 mg/kg (Gorham et al., 1990). Repeat injections after three days are necessary in some animals (Laven and Andrews, 1991; Scott, 1994). Tilmicosin is not approved for use in lactating cattle because of the prolonged period (two to three weeks) during which milk residues can be detected. Intramammary tilmicosin at drying-off has been shown to be efficacious in curing some existing *S. aureus* infection (Dingwell et al., 2003).

Tilmicosin is approved for single-dose SC treatment of ovine respiratory disease associated with *M. haemolytica*. Administration of tilmicosin may be fatal in goats.

Swine. Tilmicosin has been shown by experimental and clinical studies to be useful as an oral medication in swine (200–400 ppm) in the control of *Actinobacillus* spp. or *P. multocida* pneumonia (Paradis, 2004). It may also be useful in the control of atrophic rhinitis. In feed, treatment with 400 ppm of tilmicosin phosphate significantly reduced the presence of *A. pleuropneumoniae* on the surface of tonsils but was unable to completely eliminate the organism from deeper tonsillar tissues and to prevent bacterial shedding by carrier animals (Fittipaldi et al., 2005). There is no information on its effect against *Mycoplasma pneumoniae*. It is effective in vitro against *Lawsonia intracellularis* and would likely control proliferative enteropathy. Tilmicosin should only be administered orally to swine as intramuscular administration causes vomiting, tachypnea, convulsions and sometimes death.

Rabbits. Tilmicosin at 25 mg/kg SC was effective in treating pasteurellosis in rabbits; this dose may need to be repeated after three days to achieve a clinical cure (McKay et al., 1996).

Poultry. Tilmicosin is effective in the treatment of experimentally induced *Mycoplasma gallisepticum*

infection when administered at 50 mg/l of drinking water for three or five days (Charleston et al., 1998). At 300–500 g/ton it prevented infection; interestingly, use of the pellet binder bentonite inhibited the effect of tilmicosin in a concentration-dependent manner (Shryock et al., 1994).

Other species. Tilmicosin is not approved or recommended for use in goats, or species other than those described above, by any route, because of toxicity. It can be immediately fatal on injection in pigs.

Bibliography

- Charleston B, et al. 1998. Assessment of the efficacy of tilmicosin as a treatment for *Mycoplasma gallisepticum* infections in chickens. *Avian Pathol* 27:190.
- Dingwell RT, et al. 2003. Efficacy of intramammary tilmicosin and risk factors for cure of *Staphylococcus aureus* infection in the dry period. *J Dairy Sci.* 86:159.
- Fittipaldi N, et al. 2005. Assessment of the efficacy of tilmicosin phosphate to eliminate *Actinobacillus pleuropneumoniae* from carrier pigs. *Can J Vet Res.* 69:146.
- Gorham PE, et al. 1990. Tilmicosin as a single injection treatment for respiratory disease of feedlot cattle. *Can Vet J* 31:826.
- Laven R, Andrews AH. 1991. Long-acting antibiotic formulations in the treatment of calf pneumonia: A comparative study of tilmicosin and oxytetracycline. *Vet Rec* 129:109.
- Main BW, et al. 1996. Cardiovascular effects of the macrolide antibiotic, tilmicosin, administered alone or in combination with propranolol or dobutamine, in conscious unrestrained dogs. *J Vet Pharm Ther* 19:225.
- McKay SG, et al. 1996. Use of tilmicosin for treatment of pasteurellosis in rabbits. *Am J Vet Res* 57:1180.
- Musser J, et al. 1996. Comparison of tilmicosin with long-acting oxytetracycline for treatment of respiratory disease in calves. *J Am Vet Med Assoc* 208: 102.
- Ose EE, Tonkinson LV. 1988. Single-dose treatment of neonatal calf pneumonia with the new macrolide antibiotic tilmicosin. *Vet Rec* 123:367.
- Paradis MA. 2004. Efficacy of tilmicosin in the control of experimentally induced *Actinobacillus pleuropneumoniae* infection in swine. *Can J Vet Res* 68:7.
- Rowan TG, et al. 2004. Efficacy of danofloxacin in the treatment of respiratory disease in European cattle. *Vet Rec.* 154:585.
- Scorneaux B, Shryock TR. 1998. Intracellular accumulation, subcellular distribution and efflux of tilmicosin in swine phagocytes. *J Vet Pharm Ther* 21:257.
- Scott PR. 1994. Field study of undifferentiated respiratory disease in housed beef calves. *Vet Rec* 134:325.
- Schumann FJ, et al. 1991. Prophylactic medication of feedlot calves with tilmicosin. *Vet Rec* 128:278.
- Shen J, et al. 2005. Pharmacokinetics of tilmicosin after oral administration in swine. *Am J Vet Res.* 66:1071.
- Shryock TR, et al. 1994. Effect of bentonite incorporated in a feed ration with tilmicosin in the prevention of induced *Mycoplasma gallisepticum* airsacculitis in broiler chickens. *Avian Dis* 38:501.
- Shryock TR, et al. 1996. Minimum inhibitory concentration breakpoints and disk diffusion inhibitory interpretive criteria for tilmicosin susceptibility testing against *Pasteurella* spp. associated with bovine respiratory disease. *J Vet Diagn Invest* 8:337.
- Young C. 1995. Antimicrobial metaphylaxis for undifferentiated bovine respiratory disease. *Comp Cont Ed Pract Vet* 17:133.
- Ziv G, et al. 1995. Tilmicosin antibacterial activity and pharmacokinetics in cows. *J Vet Pharm Ther* 18:340.

Tulathromycin

Tulathromycin, a semi-synthetic macrolide, consists of a regioisomeric equilibrated mixture of a 13-membered ring (10%) and a 15-membered ring (90%) macrolide. The unique structural feature of this antimicrobial places it in a novel category of macrolides termed triamilides.

Antimicrobial Activity

The antimicrobial activity of tulathromycin appears similar to that of tilmicosin. The activity of tulathromycin against Gram-positive bacterial pathogens has not yet been studied extensively. The drug is active in vitro against many Gram-negative pathogens including *M. haemolytica*, *P. multocida*, *H. somni*, *A. pleuropneumoniae*, *Haemophilus parasuis* (MIC₉₀ 2 µg/ml), and *Bordetella bronchiseptica* (MIC₉₀ 8 µg/ml). Tulathromycin is active in vitro against some *Mycoplasma* spp. although resistance is common (Table 12.1).

Pharmacokinetic Properties

The pharmacokinetics of tulathromycin in cattle and swine are characterized by rapid absorption from the injection site, extensive distribution into tissues, and slow elimination which collectively contribute to high and sustained lung concentrations. The bioavailability of tulathromycin following SC (cattle) and IM (swine) administration is approximately 90% and the elimination half-life is about 90 hours. The apparent volume of distribution following IV administration is 12 L/kg. Peak lung concentrations are approximately 4 µg/ml. Lung concentrations are 25 to 180 times higher than concurrent serum concentrations. Lung elimination half-life values for cattle and swine are six to seven days (Nowakoski et al., 2004; Benchaoui et al., 2004).

Table 12.1. In vitro activity (MIC₉₀) of veterinary macrolides (µg/ml) against selected bacterial and mycoplasmal pathogens.

Organisms	Erythromycin	Tylosin	Spiramycin	Tilmicosin	Tulathromycin
Gram-positive aerobes					
<i>Arcanobacterium pyogenes</i>	2	2	4	0.03	> 64
<i>Erysipelothrix rhusiopathiae</i>	0.13	< 0.13	0.25	< 0.13	
<i>Rhodococcus equi</i>	≤ 0.25	64	128	32	
<i>Staphylococcus aureus</i>	0.25	2	8	1	
<i>Streptococcus agalactiae</i>	≤ 1	1		4	
<i>Streptococcus uberis</i>	≤ 0.5	1	0.5		
Gram-negative aerobes					
<i>Actinobacillus pleuropneumoniae</i>	8	32	32	2	32
<i>Histophilus somni</i>	2	8	128	8	4
<i>Mannheimia haemolytica</i>	16	128		4	2
<i>Pasteurella multocida</i>	16	128		16	1
Anaerobes					
<i>Dichelobacter nodosus</i>	0.25	1	1		
<i>Bacteroides fragilis</i>	32	0.25*	> 64		
<i>Fusobacterium necrophorum</i>	8	4	64	4	
<i>Brachyspira hyodysenteriae</i>	> 128	> 128	> 128	> 64	
<i>Clostridium perfringens</i>	4	2		4	
Mycoplasma					
<i>Mycoplasma bovis</i>	0.5	0.5	4	> 128	1
<i>Mycoplasma hyorhinis</i>	128	1	0.5	4	> 32
<i>Mycoplasma hyopneumoniae</i>	4	1	1	0.5	> 32
<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i>	0.06	0.06	0.5	0.06	
<i>Ureaplasma</i> spp.		0.13	0.5		
Other					
<i>Leptospira</i> spp.	0.06	0.06			
<i>Lawsonia intracellularis</i>	0.5	64		2	

*Some reports show resistance

Toxicity and Adverse Effects

Tulathromycin is safe to use in swine and cattle. No serious adverse events were noted during the clinical development program of the drug. At 10 times the recommended dosage, the most significant adverse effects were associated with pain, swelling and discoloration at the injection site. Safety has not been assessed in other species.

Administration and Dosage

Administration is summarized in Table 12.2.

Clinical Applications

Cattle. Tulathromycin is indicated for the treatment or control of bovine respiratory disease caused by *M. haemolytica*, *P. multocida*, or *H. somni*. In the European Union, the label claim also includes treatment and prevention of *Mycoplasma bovis* infection. In multiple studies, tulathromycin was more effective than

florfenicol or tilmicosin in the prevention or treatment of undifferentiated respiratory disease in cattle (Rooney et al., 2005; Skogerboe et al., 2005; Nutsch et al., 2005a). Tulathromycin was also effective in the treatment of calves experimentally infected with *M. bovis* (Godinho et al., 2005). Interestingly, the drug was as effective regardless of the MIC of the challenge strain (1 or >64 µg/ml).

Swine. Tulathromycin is indicated for the treatment or control of swine respiratory disease caused by *A. pleuropneumoniae*, *P. multocida*, *B. bronchiseptica*, or *H. parasuis*. In the European Union, the label claim also includes treatment and prevention of *Mycoplasma hyopneumoniae* infection. Tulathromycin was as at least as effective as ceftiofur, florfenicol or tiamulin for the treatment of undifferentiated respiratory disease in swine (McKelvie et al., 2005; Nutsch et al., 2005b). A single dose of tulathromycin was as effective as three

Table 12.2. Usual dosages of selected macrolides in animals.

Species	Drug	Dosage (mg/kg)	Route	Interval (h)
Dog/cat	Erythromycin	10-20	PO	8-12
	Clarithromycin	5-10	PO	12
	Azithromycin	5 (cat), 10 (dog)	PO	24
	Tylosin	10-20	PO	12
		5-10	IM	12
Ruminants	Spiramycin	23	PO	24
	Erythromycin	2.2-8.8	IM	24
	Tylosin	20	IM	24
	Tilmicosin ^a	10	SC	Single dose
	Spiramycin	20	IM	24
Horses	Tulathromycin	2.5	SC	Single dose
	Erythromycin	25	PO	6-8
	Erythromycin	5	IV ^b	6
	Clarithromycin	7.5	PO	12
	Azithromycin	10	PO, IV ^b	24-48
Swine	Erythromycin	2-20	IM	12-24
	Tylosin	9	IM	12-24
	Tilmicosin	200-400 g/ton of feed		
	Tulathromycin	2.5	IM	Single dose

^acattle and sheep only^bslow IV infusion

daily administrations of enrofloxacin for the treatment of pigs inoculated experimentally with *M. hyopneumoniae* (Nanjiani et al., 2005).

Bibliography

- Benchouai HA, et al. 2004. Pharmacokinetics and lung tissue concentrations of tulathromycin in swine. *J Vet Pharmacol Ther.* 27:203.
- Godinho KS, et al. 2005. Efficacy of tulathromycin in the treatment of bovine respiratory disease associated with induced *Mycoplasma bovis* infections in young dairy calves. *Vet Ther.* 6:96.
- McKelvie J, et al. 2005. Evaluation of tulathromycin for the treatment of pneumonia following experimental infection of swine with *Mycoplasma hyopneumoniae*. *Vet Ther.* 6:197.
- Nanjiani IA, et al. 2005. Evaluation of the therapeutic activity of tulathromycin against swine respiratory disease on farms in Europe. *Vet Ther.* 6:203.
- Nowakowski MA, et al. 2004. Pharmacokinetics and lung tissue concentrations of tulathromycin, a new triamylide antibiotic, in cattle. *Vet Ther.* 5:60.
- Nutsch RG, et al. 2005a. Comparative efficacy of tulathromycin, tilmicosin, and florfenicol in the treatment of bovine respiratory disease in stocker cattle. *Vet Ther.* 6:167.
- Nutsch RG, et al. 2005b. Efficacy of tulathromycin injectable solution for the treatment of naturally occurring Swine respiratory disease. *Vet Ther.* 6:214.

Rooney KA, et al. 2005. Efficacy of tulathromycin compared with tilmicosin and florfenicol for the control of respiratory disease in cattle at high risk of developing bovine respiratory disease. *Vet Ther.* 6:154.

Skogerboe TL, et al. 2005. Comparative efficacy of tulathromycin versus florfenicol and tilmicosin against undifferentiated bovine respiratory disease in feedlot cattle. *Vet Ther.* 6:180.

Other Classic Macrolides

Uncommon macrolide antibiotics (oleandomycin, josamycin, kitasamycin, rosaramicin) have activity similar to erythromycin, spiramycin, and tylosin. There is little reported experience with their use in veterinary medicine, although kitasamycin is used in Japan. The agents appear to have nothing to offer over the commonly used classic macrolide antibiotics.

Advanced Generation Macrolide Antibiotics: Roxithromycin, Dirithromycin, Clarithromycin, and Azithromycin

Interest in the macrolides has been stimulated by their activity against traditional and emerging human pathogens, including *Campylobacter* spp., *Helicobacter* spp., and *Legionella* spp., as well as against intracellu-

Table 12.3. In vitro activity (MIC₉₀) of erythromycin and newer macrolides (µg/ml) against selected bacterial pathogens.

Organisms	Erythromycin	Roxithromycin	Clarithromycin	Azithromycin
Gram-positive aerobes				
<i>Arcanobacterium pyogenes</i>	≤0.016	≤0.03	≤0.016	≤0.016
<i>Erysipelothrix rhusiopathiae</i>	0.03	0.13	0.06	0.03
<i>Listeria monocytogenes</i>	0.25	0.5	0.13	1
<i>Rhodococcus equi</i>	0.5	0.25	0.06	1
<i>Staphylococcus aureus</i>	0.25	0.25	0.25	0.25
<i>Streptococcus agalactiae</i>	0.13	0.13	0.06	0.13
<i>S. equi</i> (subsp. <i>equi</i> and <i>zooepidemicus</i>)	≤0.25		≤0.06	≤0.12
Gram-negative aerobes				
<i>Escherichia coli</i>	>4		>4	>8
<i>Klebsiella</i> spp.	>4		>4	>8
<i>Salmonella enterica</i>	>4		>4	4
<i>Pasteurella multocida</i>	4	4	2	1
<i>Pasteurella</i> spp. (equine)	1		1	0.25
<i>Brucella</i> spp.	16	16	8	2
Gram-negative: other				
<i>Bartonella henselae</i>	0.13	0.13	0.03	0.016
<i>Campylobacter</i> spp.	2	2	2	0.5
<i>Helicobacter pylori</i>	0.5	0.125	1	0.25
Anaerobes				
<i>Bacteroides fragilis</i>	>8		16	4
<i>Clostridium perfringens</i>	4		4	4
<i>Fusobacterium necrophorum</i>	16	16	8	1
<i>Peptostreptococcus</i> spp.	>32	>32	>32	>32

lar organisms which have emerged through the AIDS epidemic, such as *Bartonella* spp. and *Mycobacterium* spp. Newer erythromycin derivatives with enhanced pharmacokinetic and in some cases broader antibacterial activities include roxithromycin, dirithromycin, clarithromycin, and azithromycin. Roxithromycin is an acid-stable derivative of erythromycin with similar activity to erythromycin that is better absorbed after oral administration and has a considerably longer half-life (13 hours). It is a well tolerated alternative to erythromycin for daily oral administration. Dirithromycin has similar in vitro activity as erythromycin but offers the advantage of once-daily dosage. Clarithromycin, a 6-O-methyl derivative of erythromycin, is about twice as active as erythromycin against bacteria on a weight basis, has a half-life about twice that of erythromycin, and includes good activity against *Mycobacterium avium*. Azithromycin, an acid-stable 15-membered-ring azalide, is more active than erythromycin against Gram-negative bacteria and also has a considerably lengthened half-life relative to erythromycin. The application of these and other newer macrolides for veterinary use will likely take advantage

of their long half-lives which may provide for less frequent administration in the treatment of infections caused by pathogens such as *Mycoplasma* and *Campylobacter*, and of infections caused by intracellular bacteria.

Antimicrobial Activity

Organisms with MIC ≤2 µg/ml for newer macrolides are generally regarded as susceptible and ≥8 µg/ml as resistant. All these macrolides approved for use in human medicine share a similar antibacterial spectrum of activity against Gram-positive isolates with clarithromycin being the most active against *Rhodococcus equi* (Table 12.3). Azithromycin has the broadest in vitro activity against Gram-negative bacteria, including moderate activity against *Salmonella enterica*, but the others also have activity against important human upper respiratory tract Gram-negative pathogens (*Bordetella pertussis*, *Haemophilus influenzae*, and *Moraxella catarrhalis*). Other important antibacterial effects include excellent activity against the genera *Bartonella*, *Borrelia*, *Brucella*, *Campylobacter*, *Chlamydia* (trachomatis), *Legionella*, *Leptospira*, *Mycoplasma*, members of the *Spirochetaceae*, and *Urea-*

plasma. Mycobacteria such as *M. avium* are often moderately susceptible. Activity against anaerobic bacteria is variable (Table 12.3).

Pharmacokinetic Properties

In comparison to erythromycin, from which they have been developed, newer macrolides are acid stable, produce fewer gastrointestinal side effects, have higher bioavailability following oral administration, have considerably lengthened serum half-lives, and produce higher tissue concentrations, so that single or twice daily dosing is appropriate. Oral bioavailability of azithromycin is approximately 97% in dogs and about 50% in cats and foals. The oral bioavailability of clarithromycin in dogs is lower and ranges between 60 and 80%. Bioavailability of clarithromycin in dogs is not significantly influenced by feeding (Vilmanyi et al., 1996). Azithromycin, but not clarithromycin, is also available as an IV formulation. Serum elimination half-lives are 20 h and 35 h for azithromycin in foals and cats, respectively. The elimination half-life of clarithromycin in foals (4.8 h) is shorter than that of azithromycin but longer than that of erythromycin (1 h). The long half-lives of these newer drugs, which are particularly marked for azithromycin, apparently result from extensive uptake by, and slow release from, tissues rather than delayed metabolism. The major route of excretion is the bile and intestinal tract, although clarithromycin is more markedly excreted through the kidney. About half the administered azithromycin is excreted unchanged in the bile in dogs and cats; tissue half-lives in cats vary from 13 hours in fat to 72 hours in heart muscle (Hunter et al., 1995). Tissue concentrations of azithromycin in the lung and spleen of cats exceeded 1 µg/ml 72 hours after a single oral dose of 5.4 mg/kg (Hunter et al., 1995). Tissue concentrations of azithromycin are generally 10–100 times those achieved in serum. The extensive tissue distribution of azithromycin appears to result from its concentration within macrophages and neutrophils. The half-life of azithromycin in foal neutrophils is 49 hours (Davis et al., 2002). Bronchoalveolar cells and pulmonary epithelial lining fluid concentrations in foals are 15–170-fold and 1–16-fold higher than concurrent serum concentrations, respectively (Jacks et al., 2001). In humans, clarithromycin achieves considerably greater concentrations in pulmonary epithelial lining fluid and alveolar macrophages than either erythromycin or azithromycin. However, the half-life

of clarithromycin at these sites is much shorter than that of azithromycin.

Toxicity and Adverse Effects

Newer macrolides have few of the adverse gastrointestinal effects of erythromycin, and are well tolerated in humans. Limited experience in dogs and cats suggests the same to be true in these species. As with earlier macrolides, these drugs can induce severe enterocolitis in adult horses. Clarithromycin may be fetotoxic and should not be administered to pregnant animals.

Administration and Dosage

Dosage recommendations for dogs, cats, and foals are summarized in Table 12.2.

Clinical Applications

There is limited experience with the use of newer macrolides in veterinary medicine, but these drugs offer the advantage for monogastrates of better oral bioavailability, potentially fewer adverse effects, and once-daily dosing for a shorter time than with classical macrolides. Their particular efficacy against intracellular organisms is a significant advantage. Potential applications include those described for erythromycin. For example, erythromycin is an alternative to penicillin in penicillin-allergic animals for the treatment of infections caused by susceptible Gram-positive aerobes, an alternative to ampicillin or amoxicillin in the treatment of leptospirosis, and an alternative to tetracyclines for *Rickettsia* and *Coxiella* infections. Newer macrolides may have advantages in the treatment of intracellular infections in monogastrates, including *Bartonella*, *Chlamydophila psittaci* and atypical mycobacterial infections. Clarithromycin is effective in the treatment of atypical *Mycobacterium* infections, when combined with other antibiotics. Other areas which need to be investigated are use against *Mycoplasma* infections in animals, since medically important *Mycoplasma* are highly susceptible in vitro.

Dogs and Cats. Azithromycin in combination with atovaquone was effective in eliminating *Babesia gibsoni* from persistently infected dogs (Birkenheuer et al., 2004). Administration of azithromycin to dogs with experimental Rocky Mountain spotted fever resulted in improvement of most of the clinical signs but was not as effective as doxycycline or trovafloxacin in

decreasing vascular injury to the eye and clearing visible circulating rickettsiae (Breitschwerdt et al., 1999). Azithromycin prevented or resolved episodes of acute arthritis and reduced the bacterial load but failed to eliminate *Borrelia burgdorferi* in infected dogs (Straubinger, 2000). Azithromycin given at a dose of 10–15 mg/kg daily for 3 days and then twice weekly, provided a similar rapid resolution of clinical signs when compared to doxycycline in cats with *Chlamidophila felis* infections. However, as opposed to doxycycline, azithromycin was ineffective in clearing infection (Owen et al., 2003).

Horses. The main indication for the use of azithromycin or clarithromycin in horses is the treatment of *Rhodococcus equi* infections in foals. The combination clarithromycin-rifampin is more effective than erythromycin-rifampin or azithromycin-rifampin especially in severely affected foals (Giguère et al., 2004). The incidence of diarrhea in foals treated with clarithromycin is similar to that observed with erythromycin. In most cases, diarrhea is mild and self-limiting. However, diarrhetic foals should be monitored carefully because some may develop depression and severe diarrhea, leading to dehydration and electrolyte loss. In adult horses, clarithromycin and azithromycin, like erythromycin, should be used only when no other alternatives are available, because of the potential for severe enterocolitis.

Bibliography

- Birkenheuer AJ. 2004. Efficacy of combined atovaquone and azithromycin for therapy of chronic *Babesia gibsoni* (Asian genotype) infections in dogs. *J Vet Intern Med.* 2004 18:494.
- Breitschwerdt EB, et al. 1999. Efficacy of doxycycline, azithromycin, or trovafloxacin for treatment of experimental Rocky Mountain spotted fever in dogs. *Antimicrob Agents Chemother.* 43:813.
- Davis JL, et al. 2002. Pharmacokinetics of azithromycin in foals after i.v. and oral dose and disposition into phagocytes. *J Vet Pharmacol Ther.* 25:99.
- Giguère S, et al. 2004. Retrospective comparison of azithromycin, clarithromycin, and erythromycin for the treatment of foals with *Rhodococcus equi* pneumonia. *J Vet Intern Med.* 18:568.
- Jacks S, et al. 2001. Pharmacokinetics of azithromycin and concentration in body fluids and bronchoalveolar cells in foals. *Am J Vet Res.* 62:1870.

- Hunter RP, et al. 1995. Pharmacokinetics, oral bioavailability and tissue distribution of azithromycin in cats. *J Vet Pharm Ther* 18:38.
- Lavy E, et al. 1995. Minimal inhibitory concentrations for canine isolates and oral absorption of roxithromycin in fed and fasted dogs. *J Vet Pharm Ther* 18:382.
- Owen WM, et al. 2003. Efficacy of azithromycin for the treatment of feline chlamydophilosis. *J Feline Med Surg* 5:305.
- Straubinger RK. 2000. PCR-Based quantification of *Borrelia burgdorferi* organisms in canine tissues over a 500-day postinfection period. *J Clin Microbiol.* 38:2191.
- Vilmanyi E, et al. 1996. Clarithromycin pharmacokinetics after oral administration with or without fasting in cross-bred beagles. *J Small Anim Pract* 37:535.

Ketolides

Ketolides are members of a new semisynthetic 14-membered ring macrolide, with a 3-keto group instead of an α -L-cladinose on the erythronolide A ring. The two most widely studied ketolides are telithromycin and cethromycin. Both have been developed for oral use. Their spectrum of activity is similar to that of newer generation macrolides. However, they offer the striking advantage of overcoming current mechanisms of resistance to standard macrolides within Gram-positive cocci. Ketolides do not induce MLSB resistance and are active against bacterial isolates carrying erythromycin resistance methylase (*erm*) genes. They are also active against most isolates resistant to macrolides because of macrolide efflux (*mef*) genes. Their pharmacokinetics display a long half-life as well as extensive tissue distribution and uptake into respiratory tissues and fluids, allowing for once-daily dosing. The major indication for the use of ketolides in human medicine is the treatment of respiratory infection caused by erythromycin-resistant Gram-positive isolates. Clinical trials focusing on respiratory infections indicate bacteriological and clinical cure rates similar to comparators, even in patients infected with macrolide-resistant strains.

Bibliography

- File TM. 2005. Telithromycin new product overview. *J Allergy Clin Immunol.* 115(2):S1.
- Zhanell GG, et al. 2003. Ketolides: an emerging treatment for macrolide-resistant respiratory infections, focusing on *S. pneumoniae*. *Expert Opin Emerg Drugs.* 8:297.

Aminoglycosides

Patricia M. Dowling

The aminoglycosides are bactericidal antibiotics primarily used to treat serious infections caused by aerobic Gram-negative bacteria and staphylococci. Amikacin and tobramycin have excellent activity against *Pseudomonas aeruginosa*. However, the use of aminoglycosides has been eclipsed by the development of fluoroquinolones, which have stronger safety profiles and better distribution kinetics. Renal accumulation of aminoglycosides results in detectable drug residues for prolonged periods, so their extra-label use in food animals is discouraged. Despite this, they remain important drugs in the treatment of severe Gram-negative sepsis, although their highly cationic, polar nature means that distribution across membranes is limited. Single daily dosing is now recommended for most dosage regimens as it maximizes efficacy and reduces toxicity.

Chemistry

The aminoglycoside antibiotics—streptomycin, dihydrostreptomycin, kanamycin, gentamicin, tobramycin, amikacin, and neomycin—are large molecules with numerous amino acid groups, making them basic polycations that are highly ionized at physiological pHs. Their polarity largely accounts for the pharmacokinetic properties that are shared by all members of the group. Chemically, they consist of a hexose nucleus to which amino sugars are attached by glycosidic linkages. This is why these molecules are also referred to as aminocyclitols or aminoglycosidic aminocyclitols. The aminoglycosides can be divided into 4 groups on the basis of the type and substitution pattern of their aminocyclitol molecule: Derivatives containing the aminocyclitol streptidine (e.g. streptomycin and dihydrostreptomycin), derivatives containing the aminocyclitol streptamine (e.g. spectinomycin), derivatives

containing a 4,5-disubstituted deoxystreptamine moiety (e.g., neomycin), and derivatives containing a 4,6-disubstituted deoxystreptamine moiety (e.g., gentamicin, kanamycin, amikacin, tobramycin). They all have similar pharmacokinetic properties.

Mechanism of Action

Aminoglycosides must penetrate the bacteria to assert their effect. Penetration can be enhanced by the presence of a drug that interferes with cell wall synthesis, such as a beta-lactam antibiotic. Susceptible, aerobic Gram-negative bacteria actively pump the aminoglycoside into the cell. This is initiated by an oxygen-dependent interaction between the antibiotic cations and the negatively charged ions of the bacterial membrane lipopolysaccharides. This interaction displaces divalent cations (Ca^{++} , Mg^{++}), which effects membrane permeability. Once inside the bacterial cell, aminoglycosides bind to the 30S ribosomal sub-unit and cause a misreading of the genetic code, interrupting normal bacterial protein synthesis. This leads to changes in cell membrane permeability, resulting in additional antibiotic uptake, further cell disruption, and ultimately, cell death.

The extent and type of misreading vary because different members of the group interact with different proteins. Streptomycin acts at a single site but the other drugs act at several sites. Other effects of aminoglycosides include interference with the cellular electron transport system, induction of RNA breakdown, inhibition of translation, effects on DNA metabolism, and damage to cell membranes. The bactericidal effect is through the formation of abnormal cell membrane channels by misread proteins.

Aminoglycoside action is bactericidal and dose (concentration) dependent. For example, gentamicin

concentrations in the range of 0.5–5.0 µg/ml are bactericidal for Gram-positive and some Gram-negative bacteria. At 10–15 µg/ml, gentamicin is effective against the more resistant bacteria such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus mirabilis*. The clinical implication is that high initial doses increase ionic bonding, which enhances the initial concentration-dependent phase of rapid antibiotic internalization and leads to greater immediate bactericidal activity. Human clinical studies demonstrate that proper initial therapeutic doses of aminoglycosides are critical in reducing mortality from Gram-negative sepsis. For antimicrobials whose efficacy is concentration-dependent, high plasma concentration levels relative to the MIC of the pathogen (C_{max}:MIC ratio, also known as the inhibitory quotient or IQ) and the area under the plasma concentration-time curve that is above the bacterial MIC during the dosage interval (area under the inhibitory curve, AUIC = AUC/MIC) are the major determinants of clinical efficacy. For the aminoglycosides, a C_{max}:MIC ratio of 10 is suggested to achieve optimal efficacy (McKellar et al., 2004).

The aminoglycosides have a significant Post-Antibiotic Effect (PAE); the period of time where antimicrobial concentrations are below the bacterial MIC, but the antimicrobial-damaged bacteria are more susceptible to host defenses (Gilbert, 1991). The duration of the PAE tends to increase as the initial aminoglycoside concentration increases.

Antimicrobial Activity

The antibacterial action of the aminoglycosides is directed primarily against aerobic, Gram-negative bacteria. Because bacterial uptake is oxygen-dependent, they are not active against facultative anaerobes or aerobic bacteria under anaerobic conditions. They are active against some Gram-positive bacteria, such as *Staphylococcus* spp. They are often effective against enterococci, but therapy against streptococci is more effective when combined with a beta-lactam antibiotic. *Salmonella* and *Brucella* spp. are intracellular pathogens and are often resistant. Some mycobacteria, spirochetes, and *Mycoplasma* spp. are susceptible. In potency, spectrum of activity, and stability to enzymes from plasmid-mediated resistance, amikacin > tobramycin ≥ gentamicin > neomycin = kanamycin > streptomycin. Amikacin was developed from kanamycin and has the broadest spectrum of activity of the aminoglycosides. It is effective against Gram-negative

strains not susceptible to other aminoglycosides because it is more resistant to bacterial enzymatic inactivation. It is also considered the least nephrotoxic, but it is less active against streptococci than gentamicin. Streptomycin and dihydrostreptomycin are the most active of these drugs against mycobacteria and *Leptospira* spp., and the least active against other organisms. The activity of selected aminoglycosides against selected bacteria and *Mycoplasma* is shown in Table 13.1.

The bactericidal action of these agents on aerobic Gram-negative bacteria is markedly influenced by pH, being most active in an alkaline environment. Increased local acidity secondary to tissue damage or bacterial destruction may account for failure of aminoglycosides to kill usually susceptible microorganisms. Another factor affecting activity is the presence of purulent debris, which binds to aminoglycosides and inactivates them. When using an aminoglycoside to treat infections with purulent debris, surgical debridement and/or drainage increases efficacy.

Resistance to Aminoglycoside Antibiotics

Most clinically important resistance to aminoglycosides is caused by R-plasmid-specified enzymes, broadly classified as phosphotransferases, acetyltransferases, and adenylyltransferases (Table 13.2). At least 11 enzymes have been identified which can inactivate aminoglycosides. These enzymes modify the aminoglycosides at their exposed hydroxyl or amino groups to prevent ribosomal binding. They are present in the periplasmic space of bacteria, so that extracellular inactivation of drug does not occur. Plasmid-mediated resistance to the aminoglycosides is transferable between bacteria. A single type of plasmid may confer cross-resistance to multiple aminoglycosides and to other unrelated antimicrobials. A single bacterial isolate may have any one of a variety of combinations of resistance to different antibiotics conferred by the particular plasmid it carries. For example, one *E. coli* strain may be simultaneously resistant to ampicillin, apramycin, chloramphenicol, gentamicin, kanamycin, sulfonamide, streptomycin, tetracycline, and trimethoprim (Pohl et al., 1993). Antimicrobial resistance in organisms such as *E. coli* and *Salmonella* species is a focus of international research due to potential transference of antimicrobial resistance from animal to human pathogens.

Strains with reduced permeability and consequently

Table 13.1. Activity (MIC₉₀) of selected aminoglycosides (µg/ml) against common bacterial pathogens.

Organism	Streptomycin	Neomycin	Kanamycin	Gentamicin
Gram-positive cocci				
<i>Staphylococcus aureus</i>	32	0.5	4	1
<i>Streptococcus agalactiae</i>	128		128	64
<i>S. uberis</i>	64	32	32	
Gram-positive rods				
<i>Arcanobacterium pyogenes</i>	>128	128	64	8
<i>Bacillus anthracis</i>	??8		0.5-4	≤4
<i>Corynebacterium pseudotuberculosis</i>				4
<i>C. renale</i>	64		2	≤0.25
<i>Erysipelothrix rhusiopathiae</i>			>64	≤64
<i>Listeria monocytogenes</i>	32	4	16	16
<i>Mycobacterium tuberculosis</i>	0.5			
<i>Nocardia asteroides</i>	16	4	128	16
<i>Rhodococcus equi</i>	4	≤0.25	2	≤0.25
Gram-negative rods				
<i>Actinobacillus</i> spp.	≤1		4	1
<i>Bordetella bronchiseptica</i>	256		8-16	2
<i>Brucella canis</i>	0.25		0.5	0.12
<i>Campylobacter jejuni</i>			4	0.5
<i>Escherichia coli</i>	>64	>64	>64	2
<i>Histophilus somni</i>	8	16-32	8	8
<i>Helicobacter pylori</i>			2	
<i>Klebsiella pneumoniae</i>	256	256	8	4
<i>Leptospira</i> spp.	0.5		4	
<i>Moraxella bovis</i>	16	0.12	0.12	0.5
<i>Mannheimia haemolytica</i>	>128	32	32	8
<i>Pasteurella multocida</i>				
Cattle	>128	32	32	8
Pigs	16-32		8-16	8
<i>Proteus</i> spp.	>16	16	4	2
<i>Pseudomonas aeruginosa</i>	>16	64	128	8
<i>Salmonella</i> spp.	>128	4	>32	8
<i>Taylorella equigenitalis</i>	>128	2	1	0.5

Note: Some reports are higher because of resistance. This table is designed partly to illustrate the differences in quantitative susceptibility among different aminoglycosides.

two-to fourfold increases in MIC may be selected during treatment with aminoglycosides. Such strains show cross-resistance to all other drugs within the group. Chromosomal mutation resulting in resistance is relatively unimportant except for streptomycin and dihydrostreptomycin, where it occurs readily, even during treatment, as a result of a single-step mutation to high-level resistance. For the other drugs, chromosomal resistance develops slowly, because there are many 30S ribosomal binding sites. Resistance to aminoglycosides is increasingly limiting their effectiveness. Interestingly, the ultimate source of aminoglycoside resistance genes is probably also the microorganism source of aminoglycosides in nature,

particularly *Streptomyces* spp. (Davis and Wright, 1997).

Both subinhibitory and inhibitory aminoglycoside concentrations produce resistance in bacterial cells surviving the initial ionic binding (Barclay et al., 2001). This first-exposure adaptive resistance is due to decreased aminoglycoside transport into the bacteria. Exposure to one dose of an aminoglycoside is sufficient to produce resistant variants of an organism with altered metabolism and impaired aminoglycoside uptake. In vitro, animal and clinical studies show that the resistance occurs within 1–2 hours of the first dose. The duration of adaptive resistance relates directly to the half-life of elimination of the aminoglycoside.

Table 13.2. Aminoglycoside- and aminocyclitol-modifying enzymes in R plasmid-bearing bacteria.

Modification	Enzyme	Substrates
Aminoglycoside acetyltransferase (AAC)	AAC(3)-I*	Gm
	AAC(3)-II	Gm, Tm
	AAC(3)-III	Gm, Km, Nm, Pm, Tm
	AAC(3)-IV	Gm, Km, Nm, Tm, Ak, Ap
	AAC(2')	Gm, Nm
	AAC(6')-I	Km
Aminoglycoside adenylyltransferase (AAD)	AAD(2'')	Gm, Km
	AAD(4')	Km, Tm, Nm, Pm, Ak
	AAD(4',4'')	Km, Nm, Tm
	AAD(3'')	Sm, Sp
	AAD(6)	Sm
Aminoglycoside phosphotransferase (APH)	APH(3')-I	Km, Nm, Pm
	APH(3')-III	Km, Nm, Pm
	APH(2'')	Gm, Km, Tm
	APH(3'')	Sm
	APH(6)	Sm

AK, amikacin; AP, apramycin; Gm, gentamicin; Km, kanamycin; Nm, neomycin; Pm, paramomycin; Sm, streptomycin; Sp, spectinomycin; Tm, tobramycin.

*Where enzymes act at the same position but differ in the substrates they degrade, they are shown with different Roman numerals. Position of enzyme activity on aminoglycoside is indicated by Arabic numerals in parentheses.

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With normal aminoglycoside pharmacokinetics, the resistance may be maximal for up to 16 hours after a single dose, followed by partial return of bacterial susceptibility at 24 hours and complete recovery at 40 hours. The clinical significance of this phenomenon is that frequent dosing or constant infusion of an aminoglycoside is less effective than high-dose, once-daily dosing.

Pharmacokinetic Properties

Aminoglycosides are poorly absorbed from the normal gastrointestinal tract, but are well absorbed after IM or SC injection. Following parenteral administration, effective concentrations are obtained in synovial, perilymph, pleural, peritoneal, and pericardial fluid. When given to neonates or animals with enteritis, oral absorption may be significantly increased and may result in violative tissue residues in food animals. When given by intrauterine or intramammary infusion to cows, gentamicin is well absorbed and results in prolonged tissue residues. Aminoglycosides bind to a low

extent to plasma proteins (less than 25%). As they are large molecules and highly ionized at physiological pHs, they are poorly lipid-soluble and have limited capacity to enter cells and penetrate cellular barriers. These drugs do not readily attain therapeutic concentrations in transcellular fluids, particularly cerebrospinal and ocular fluid. The milk-to-plasma equilibrium concentration ratio is approximately 0.5. Their apparent volumes of distribution are relatively small (< 0.35 L/kg) and their plasma elimination half-lives are short (1–2 hours) in domestic animals. Even though these drugs have a small volume of distribution, selective binding to tissues, including kidney cortex, occurs, so that kidney residues persist in animals for extensive periods.

Gentamicin is distributed into synovial fluid in normal horses; local inflammation may increase drug concentrations in the joint, as may repeated doses. Regional perfusion techniques and aminoglycoside-impregnated polymethyl methacrylate beads are excellent methods of local delivery that avoid the adverse effects of systemic therapy.

Elimination is entirely by renal excretion (glomerular filtration), and unchanged drug is rapidly excreted in the urine. Impaired renal function slows excretion and makes it necessary to adjust the dosage interval to prevent accumulation and toxicity. The significant individual variation in pharmacokinetic parameters among animals of the same species exacerbates problems of toxicity with this drug class (Brown et al., 1991).

Drug Interactions

Aminoglycosides are commonly additive and sometimes synergistic with beta-lactam drugs. Synergism does not usually occur in the presence of high-level plasmid-mediated or chromosomal resistance. The aminoglycosides are synergistic against streptococci, enterococci, *Pseudomonas* and other Gram-negative bacteria if combined with beta-lactam antibiotics, due to disruption of the bacterial cell wall by the beta-lactam antibiotic (Winstanley et al., 1989). Combinations of newer beta-lactam drugs with newer aminoglycosides provide optimal therapy in seriously ill, neutropenic patients with bacterial infections. Aminoglycosides are physically incompatible with a number of drugs including many beta-lactams, so they should never be mixed in the same syringe. If administered sequentially through an infusion set, care should be taken to flush well between drugs.

Table 13.3. Relative risks of toxicity of different aminoglycosides at usual dosage.

Drug	Vestibular Toxicity	Cochlear Toxicity	Renal Toxicity
Streptomycin	+++	++	(+)
Dihydrostreptomycin	++	+++	(+)
Neomycin	+	+++	+++
Kanamycin	+	++	++
Amikacin	(+)	+	++
Gentamicin	++	+	++
Tobramycin	(+)	(+)	(+)

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Toxicity and Adverse Effects

All aminoglycosides can cause varying degrees of ototoxicity and nephrotoxicity (Table 13.3). Nephrotoxicity (acute tubular necrosis) is the most common adverse effect of aminoglycoside therapy. Neomycin is the most nephrotoxic and streptomycin and dihydrostreptomycin are the least nephrotoxic. Amikacin is often recommended in critical patients over gentamicin, as it is considered less nephrotoxic.

Uptake and accumulation of aminoglycosides into renal tubular epithelium demonstrates saturable kinetics. The aminoglycosides enter the renal tubule after filtration through the glomerulus. From the luminal fluid, the cationic aminoglycoside molecules bind to anionic phospholipids on the proximal tubular cells. The aminoglycoside is taken into the cell via carrier-mediated pinocytosis and translocated into cytoplasmic vacuoles, which fuse with lysosomes. The drug is sequestered unchanged in the lysosomes. With additional pinocytosis, drug continues to accumulate within the lysosomes. The accumulated aminoglycoside interferes with normal lysosomal function and eventually the overloaded lysosomes swell and rupture. Lysosomal enzymes, phospholipids, and the aminoglycoside are released into the cytosol of the proximal tubular cell, disrupting other organelles and causing cell death (Brown et al., 1991) (Figure 13.1).

The risk factors for aminoglycoside toxicity include prolonged therapy (>7–10 days), multiple doses per day, acidosis and electrolyte disturbances (hypokalemia, hyponatremia), volume depletion (shock, endotoxemia), concurrent nephrotoxic drug therapy, age (neonates, geriatrics), pre-existing renal disease and elevated trough concentrations (Mattie et al., 1989).

Calcium supplementation reduces the risk of nephrotoxicity. Nephrotoxicity can also be decreased by feeding the patient a high protein/high calcium diet (such as alfalfa to large animals and diets higher than 25% protein to small animals), as protein and calcium cations compete with aminoglycoside cations for binding to renal tubular epithelial cells (Behrend et al., 1994; Schumacher et al., 1991). High dietary protein also increases glomerular filtration rate and renal blood flow, thereby reducing aminoglycoside accumulation.

Because nephrotoxicity is related to aminoglycoside accumulation in the renal proximal tubular cells, it is logical that peak concentrations are not related to toxicity and that longer dosage intervals result in less total drug contact with the renal brush border membrane. High-dose, once-daily dosing of aminoglycosides is now common in human and veterinary medicine; it takes advantage of the concentration-dependent killing and long PAE of these drugs and avoids first-exposure adaptive resistance and nephrotoxicity (Gilbert, 1991).

Serum concentrations of aminoglycosides can be monitored to reduce toxicity and to confirm therapeutic concentrations (Bucki et al., 2004). To allow for the distribution phase, blood sampling for the peak concentration is done 0.5–1 hour after administration and the trough sample is usually taken prior to the next dose. The peak and trough concentrations can then be used to estimate the elimination half-life for the individual patient. An increase in the elimination half-life during therapy is a very sensitive indicator of early tubular insult. If using a once-daily regimen, a blood sample just prior to the next dose will be well below the recommended trough concentrations and may even be below the limit of detection of the assay. For these patients, an 8-hour post-dose sample will provide a more accurate estimate of the elimination half-life. Serum concentrations of drug should be 0.5–2 µg/ml before the next dosage (gentamicin, tobramycin) or less than 6 µg/ml for amikacin.

If therapeutic drug monitoring is unavailable, then nephrotoxicity is detected by an increase in urine gamma glutamyl transferase (GGT) enzyme and an increase in the urine GGT:urine creatinine (Cr) ratio (van der Harst et al., 2005). The UGGT:UCr may increase to two to three times baseline within three days of a nephrotoxic dose. If these tests are not available, the development of proteinuria is the next best indica-

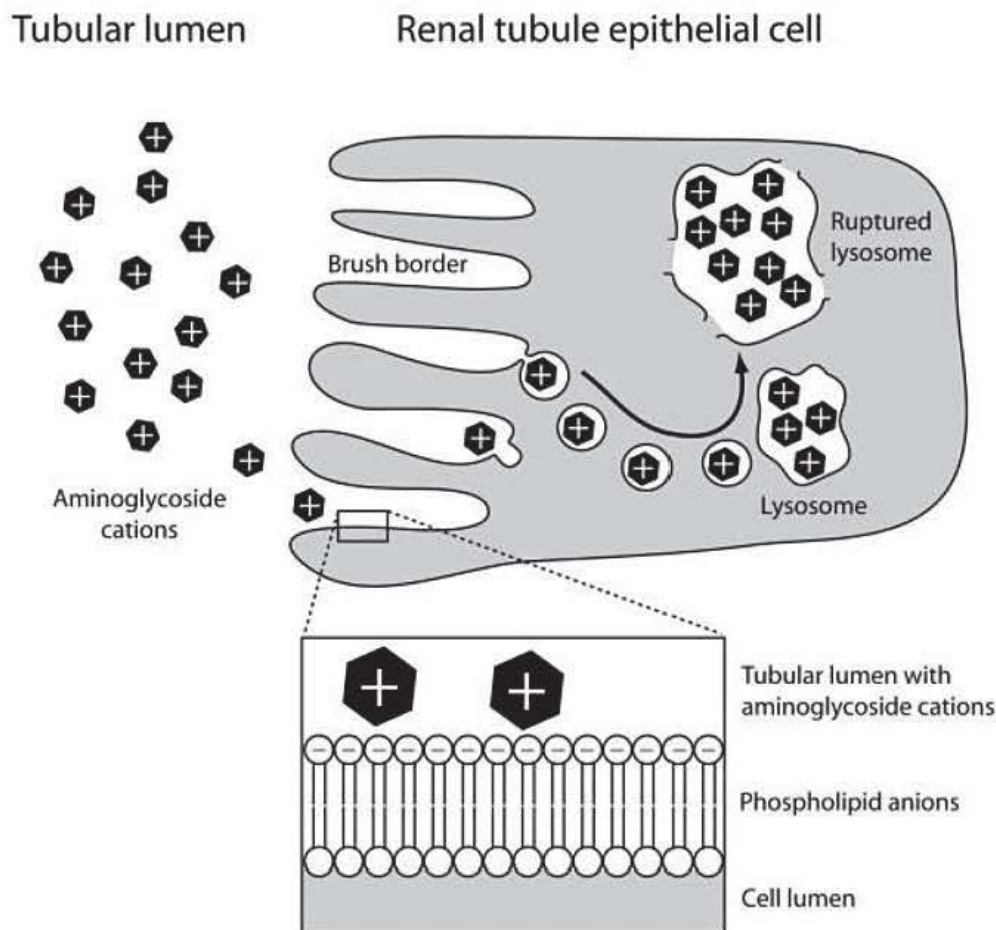


Figure 13.1. Aminoglycoside nephrotoxicity: Drug cations interact with phospholipid anions on the brush border of renal tubular epithelial cells. They are then pinocytosed and accumulate in lysosomes until they cause lysosomal rupture, which destroys the cell.

tor of nephrotoxicity and is easily determined in a practice setting. Elevations in serum urea nitrogen and Cr confirm nephrotoxicity, but are not seen for seven days after significant renal damage has occurred. Elimination half-lives of 24 to 45 hours have been reported in horses with renal toxicity, further prolonging the toxic exposure to the drug. While peritoneal dialysis is useful in lowering creatinine and serum urea nitrites, it may not be effective in significantly increasing the elimination of the accumulating aminoglycoside. The animal's ability to recover most likely depends on the type of medication exposure and the amount of healthy renal tissue remaining to compensate.

Aminoglycoside ototoxicity occurs from the same mechanisms as nephrotoxicity. The tendency to produce vestibular damage (streptomycin, gentamicin) or cochlear damage (amikacin, kanamycin, neomycin)

varies with the drug. Tobramycin appears to affect both vestibular (balance) and cochlear (hearing) functions equally. This drug-specific toxicity may be due to the distribution characteristics of each drug and concentration achieved in each sensory organ. The ototoxic effect of aminoglycosides is potentiated by the loop diuretics furosemide and ethacrynic acid and probably other diuretic agents.

All aminoglycosides given rapidly IV cause bradycardia, reduce cardiac output, and lower blood pressure through an effect on calcium metabolism. These effects are of minor significance (Hague et al., 1997). Neuromuscular blockade is a rare effect, related to blockade of acetylcholine at the nicotinic cholinergic receptor. Neuromuscular blockade most often seen when anaesthetic agents are administered concurrently with aminoglycosides. Affected patients should

be treated promptly with parenteral calcium chloride at 10 to 20 mg/kg IV or calcium gluconate at 30 to 60 mg/kg IV or neostigmine at 100 to 200 µg/kg to reverse dyspnea from muscle response depression. Edrophonium at 0.5 mg/kg IV will also reverse neuromuscular blocking effects.

Dosage Considerations

Aminoglycosides produce rapid, concentration-dependent killing of Gram-negative aerobes and a prolonged post-antibiotic effect (PAE) (McKellar et al., 2004). Therefore, the maximum plasma concentration (C_{max}) to MIC ratio determines efficacy. A C_{max} :MIC ratio of 8–12:1 optimizes bactericidal activity. Higher initial serum concentrations may also be associated with a longer PAE. Traditionally, aminoglycosides were administered every 8–12 hours. If the aminoglycoside is dosed multiple times a day or the drug concentration remains constant, as with a continuous infusion, first-exposure adaptive resistance persists and increases. Dose administration at 24 hour intervals, or longer, may increase efficacy by allowing time for adaptive resistance to reverse. Some clinicians have expressed reservations about once-daily dosing when intestinal damage allows continued exposure to bacteria that may replicate during the prolonged periods of subtherapeutic aminoglycoside concentrations, but this has not been documented clinically. Studies in human and veterinary patients support high-dose, once-daily therapy with aminoglycosides (Albarelos et al., 2004; Godber et al., 1995; Magdesian et al., 1998; Martin-Jimenez et al., 1998; Nestaas et al., 2005). However, the optimal doses and the ideal therapeutic drug monitoring strategy are still unknown. All dosage regimens should take into account the patient's kidney function, the exclusive renal excretion route of aminoglycosides, and their toxicity potential. Neonates typically have a higher percentage of extracellular water than adults; therefore, the volume of distribution of aminoglycosides is higher and they typically require higher dosages.

Clinical Usage

The toxicity of aminoglycosides has largely restricted their use to the treatment of severe infections. The more toxic aminoglycosides (neomycin) are largely restricted to topical or oral use for the treatment of infections caused by Enterobacteriaceae. The less toxic aminoglycosides are usually reserved for the parenteral treatment of severe sepsis caused by Gram-

negative aerobes. Of these, gentamicin is usually the first choice followed by amikacin, which due to expense is reserved for sepsis caused by organisms resistant to gentamicin. But even the expensive aminoglycosides can be used for local therapy of musculoskeletal infections: Antimicrobial-impregnated polymethyl methacrylate beads and regional perfusion (intravenous or intraosseous) provide high local concentrations with less expense and less risk of systemic toxicity. Spectinomycin is used for treatment of systemic infections in food animals but, like streptomycin, its use is limited by resistance.

Because aminoglycoside residues persist in renal tissues for prolonged periods, their extra-label use in food animals should be avoided when there are no established scientific data on residue depletion. A voluntary resolution against the extra-label administration of aminoglycosides has been adopted by the American Association of Bovine Practitioners, the Academy of Veterinary Consultants, the National Cattlemen's Beef Association and the American Veterinary Medical Association.

Bibliography

- Albarelos G, et al. 2004. Multiple once-daily dose pharmacokinetics and renal safety of gentamicin in dogs. *J Vet Pharmacol Ther* 27:21.
- Barclay ML, et al. 2001. Aminoglycoside adaptive resistance: importance for effective dosage regimens. *Drugs* 61:713.
- Behrend EN, et al. 1994. Effects of dietary protein conditioning on gentamicin pharmacokinetics in dogs. *J Vet Pharmacol Ther* 17:259.
- Brown SA, Riviere JE. 1991. Comparative pharmacokinetics of aminoglycoside antibiotics. *J Vet Pharm Ther* 14:1.
- Bucki EP, et al. Pharmacokinetics of once-daily amikacin in healthy foals and therapeutic drug monitoring in hospitalized equine neonates. *J Vet Intern Med* 18:728.
- Davis J, Wright GD. 1997. Bacterial resistance to aminoglycoside antibiotics. *Trends Microbiol* 5:234.
- Gilbert DN. 1991. Once-daily aminoglycoside therapy. *Antimicrob Agents Chemother* 35:399.
- Godber LM, et al. 1995. Pharmacokinetics, nephrotoxicosis, and in vitro antibacterial activity associated with single versus multiple (three times) daily gentamicin treatments in horses. *Am J Vet Res* 56:613.
- Hague BA, et al. 1997. Effects of high-dose gentamicin sulfate on neuromuscular blockade in halothane-anesthetized horses. *Am J Vet Res* 58:1324.
- Leitzke S, et al. 1998. Rationale for and efficacy of prolonged-interval treatment using liposome-encapsulated amikacin in experimental *Mycobacterium avium* infection. *Antimicrob Agents Chemother* 42:459.

- Magdesian KG, et al. 1998. Pharmacokinetics of high dose of gentamicin administered intravenously and intramuscularly to horses. *J Am Vet Med Assoc* 213:1007.
- Martin-Jimenez T, et al. 1998. Population pharmacokinetics of gentamicin in horses. *Am J Vet Res* 159:1589.
- Mattie H, et al. 1989. Determinants of efficacy and toxicity of aminoglycosides. *J Antimicrob Chemother* 24:281.
- McKellar QA, et al. 2004. Pharmacokinetic/pharmacodynamic relationships of antimicrobial drugs used in veterinary medicine. *J Vet Pharmacol Ther* 27:503.
- Mitsuhashi S, Kawabe H. 1982. Aminoglycoside antibiotic resistance in bacteria. In: Whelton A, New HC, eds. *The Aminoglycosides: Microbiology, Clinical Use, and Toxicology*. New York: Marcel Dekker.
- Nestaas E, et al. 2005. Aminoglycoside extended interval dosing in neonates is safe and effective: a meta-analysis. *Arch Dis Child Fetal Neonatal Ed* 90:F294.
- Pilloud M. 1983. Antibiotiques et Chimiothérapies—de la recherche à la pratique. VI. Schweiz Arch Tierheilkd 125:301.
- Pohl P, et al. 1993. Replicon typing characterization of plasmids encoding resistance to gentamicin and apramycin in *Escherichia coli* and *Salmonella typhimurium* isolated from human and animal sources in Belgium. *Epidemiol Infect* 111:229.
- Schumacher J, et al. 1991. Effect of diet on gentamicin-induced nephrotoxicosis in horses. *Am J Vet Res* 52:1274.
- Van der Harst MR, et al. 2005. Gentamicin Nephrotoxicity—a comparison of in vitro findings with in vivo experiments in equines. *Vet Res Commun* 29:247.
- Winstanley TG, Hastings JG. 1989. Penicillin-aminoglycoside synergy and post-antibiotic effect for enterococci. *J Antimicrob Chemother* 23:189.

Streptomycin/Dihydrostreptomycin

Streptomycin and dihydrostreptomycin are members of the streptidine group. Dihydrostreptomycin has very similar properties to streptomycin but is more likely to cause deafness. Streptomycin was the earliest aminoglycoside introduced for clinical use.

Antimicrobial Activity

Streptomycin and dihydrostreptomycin are active against mycobacteria, some *Mycoplasma* spp., some Gram-negative rods (including *Brucella*), and some *Staphylococcus aureus*. With the exception of its action on mycobacteria, streptomycin is the least active of the aminoglycosides. Among susceptible bacteria are *Leptospira*, *Francisella tularensis*, *Yersinia pestis*, and most *Campylobacter fetus* ssp. *venerealis* (Table 13.1). Organisms with MIC ≤ 4 $\mu\text{g/ml}$ are regarded as susceptible.

Antimicrobial Resistance

Acquired resistance to streptomycin and dihydrostreptomycin is widespread in veterinary pathogens and has virtually nullified the use of these drugs except for special applications. Most clinically important resistance is caused by plasmid-specified enzymes, certain of which specifically inactivate only streptomycin (Table 13.2). Plasmid-mediated resistance is commonly linked with sulfonamide, ampicillin, and tetracycline resistance genes. Chromosomal mutations to resistance arise commonly in vitro and often in vivo within a few days of treatment, although such mutants are sometimes less viable than their parents.

Drug Interactions

Streptomycin or dihydrostreptomycin are commonly combined with other drugs either to prevent the emergence of chromosomal resistance or for a synergistic effect. They are commonly synergistic with cell wall active antibiotics such as penicillin, and combination formulations were once available. This synergism occurs against Gram-positive bacteria such as streptococci, which are otherwise impermeable to the drug, and in bacteria with chromosomal mutation to low-level resistance. Synergism does not usually occur in the presence of high-level plasmid or chromosomal resistance or in Gram-negative bacteria.

Toxicity and Adverse Effects

Besides resistance, toxicity limits the use of streptomycin and dihydrostreptomycin. They cause vestibular damage—an effect that increases with the daily and cumulative dose, with the height of peak serum concentrations, and with pre-existing renal disease. In general, no toxic effects occur if streptomycin is used at recommended doses for up to 1 week. Streptomycin can cause permanent vestibular damage, producing ataxia that progresses to incoordination, nystagmus, loss of righting reflex, and death. The effects are dose-related. Daily IM injection of doses 5–10 times those recommended produces this effect in cats in about 10 days. Cats are particularly sensitive to streptomycin and usual doses may produce nausea, vomiting, salivation, and ataxia.

Neuromuscular blockade is produced when streptomycin is given at high dosage. Although this effect is insignificant at normal doses, deaths have occurred in dogs and cats given penicillin-streptomycin combina-

Table 13.4. Dosages of aminoglycosides and aminocyclitols in animals.

Drug	Route	Dosage (mg/kg or as stated)	Interval (hrs) x Duration	Comments
Amikacin	IU	2 g	24 x 3 days	Metritis in mares.
	IV, IM, SC	10	24 x 5-7 days	Gram-negative infections in adult horses.
	IV, IM, SC	20-25	24 x 5-7 days	Gram-negative infections in neonatal foals.
Apramycin	PO	12.5	24 x 7 days	Colibacillosis in swine.
	IM	20	12-24 x 5 days	Salmonellosis in calves.
Dihydrostreptomycin	IM	12.5-15	24 x 3-5 days	Leptospirosis in cattle, swine and dogs.
Gentamicin	IU	2.0-2.5 g	24 x 3-5 days	Metritis in mares.
	IV, IM, SC	4-6	24 x 5-7 days	Gram-negative infections in adult horses.
	IV, IM, SC	10-14	24 x 5-7 days	Gram-negative infections in neonatal foals.
Kanamycin	PO	10	8 x 5 days	Enteric infections in dogs.
Neomycin	PO	4.5-12	24 x 3-14 days	Enteric infections. Efficacy limited due to resistance.
Spectinomycin	PO	20-40	24 x 3-5 days	Colibacillosis in swine, chronic respiratory disease in chickens.
	SC	11-22	Once	Fowl cholera in turkeys.
	SC	10	24 x 3-5 days	Bovine respiratory disease.
Spectinomycin with lincomycin	SC	20	12-24 x 3-21 days	Bacterial infections in dogs and cats.
Streptomycin	PO	10 mg/kg	24 x 3-5 days	Enteric infections in chickens, swine and calves. Efficacy limited due to resistance.

tions for prophylaxis of surgical infection after general anesthesia, since general anesthetics and muscle relaxants potentiate the neuromuscular blocking effects. Such fatal effects have generally followed overdosing.

Administration and Dosage

Streptomycin is only available in the United States as an oral sulfate solution administered in drinking water. Dihydrostreptomycin injection is available in Canada for intramuscular injection. In the United States, dihydrostreptomycin is only available as an intramammary formulation with penicillin. In Europe, streptomycin is available as an injectable product alone and in combination with dihydrostreptomycin. Dosages of streptomycin and dihydrostreptomycin are shown in Table 13.4.

Clinical Applications

Dihydrostreptomycin or streptomycin is used in the treatment of leptospirosis in cattle, swine and dogs. Streptomycin is rarely used alone for infections in animals because of widespread resistance, particularly in Gram-negative bacteria, and the penicillin combination products are no longer available. The newer aminoglycosides are more active against a greater number of organisms and are less toxic.

Cattle, Sheep, and Goats

Leptospirosis in cattle can be successfully treated by administration of dihydrostreptomycin-penicillin G (Alt et al., 2001). Oxytetracycline, tilmicosin, and cefotiofur also were effective for resolving leptospirosis and may be useful substitutes for dihydrostreptomycin, as it is no longer available for use in food-producing animals in the United States as a parenteral product. An experimental and a field study showed that either single or 5-day streptomycin treatment in cows experimentally infected with *L. hardjo* was effective in stopping shedding for at least 70 days after treatment (Gerritsen et al., 1993; Gerritsen et al., 1994).

Swine

Dihydrostreptomycin/penicillin G is effective for treatment of acute and persistent leptospirosis in swine when given at higher-than-label doses (Alt and Bolin, 1996). This regimen may be useful for treatment of breeding stock or animals destined for import/export.

Dogs and Cats

There seems to be little place for streptomycin in infections of dogs. Flores-Castro and Carmichael (1978)

described the generally effective treatment of canine brucellosis with combined treatment with streptomycin, 10 mg/kg for 7 days, and minocycline, 15 mg/kg for 14 days. Streptomycin should not be used in cats.

Poultry

Streptomycin is sometimes used in oral treatment of nonspecific enteritis in chickens and, combined with penicillin, in the parenteral treatment of erysipelas in turkeys.

Bibliography

- Alt DP, et al. 2001. Evaluation of antibiotics for treatment of cattle infected with *Leptospira borgpetersenii* serovar *hardjo*. J Am Vet Med Assoc 219:636.
- Alt DP, Bolin CA. 1996. Preliminary evaluation of antimicrobial agents for treatment of *Leptospira interrogans* serovar *pomona* infection in hamsters and swine. Am J Vet Res 57:59.
- Flores-Castro R, Carmichael LE. 1978. Canine brucellosis: Current status of methods for diagnosis and treatment. Gaines Vet Symp 27:17.
- Gerritsen MJ, et al. 1993. Effect of streptomycin treatment on the shedding and the serologic responses to *Leptospira interrogans* serovar *hardjo* subtype *hardjobovis* in experimentally infected cows. Vet Microbiol 38:129.
- Gerritsen MJ, et al. 1994. Effective treatment with dihydrostreptomycin of naturally infected cows shedding *Leptospira interrogans* serovar *hardjo* subtype *hardjobovis*. Am J vet Res 55:339.

Dihydrostreptamine Aminoglycosides: Neomycin Group

Neomycin is the isomeric mixture of neomycins B and C. Framycetin is identical to neomycin B. Paramomycin (aminosidine) is closely related to neomycin. It is mainly used in veterinary medicine for treatment of cryptosporidiosis and leishmaniasis.

Antimicrobial Activity

Neomycin has similar activity to kanamycin on a weight basis and is several times more active than streptomycin; it is less active than gentamicin, tobramycin, and amikacin. Activity against *Staphylococcus aureus* is good but is generally low against other Gram-positive bacteria (Table 13.1). Many opportunist Gram-negative pathogens are susceptible to

neomycin, although the prevalence of susceptible strains is slightly less than for kanamycin and far less than for gentamicin. Bacteria with an MIC ≤ 8 $\mu\text{g/ml}$ are regarded as susceptible.

Resistance

Plasmid-mediated resistance occurs through a variety of enzymes (Table 13.2). Such resistance, which is often multiple, is relatively common in enteric commensals and pathogens but less common among other opportunist pathogens. Chromosomal mutation to resistance is unimportant.

Drug Interactions

Neomycin shows in vitro synergistic activity with beta-lactam antibiotics and bacitracin against Gram-positive bacteria, and it is commonly included in topical and intramammary products. The combination of EDTA-Tris and neomycin is synergistic against the microorganisms associated with otitis externa in dogs (Sparks et al., 1994).

Toxicity and Adverse Effects

Neomycin is the most toxic of the aminoglycosides and readily causes nephrotoxicity and deafness. For this reason, it should never be used parenterally. Toxic effects are generally not produced when administered orally or applied locally, but severe side effects of deafness and tubular necrosis have occurred in humans after oral administration.

Cats given high IM doses (100 mg/kg) daily showed nephrotoxic effects and became deaf in a few days; dogs are about equally susceptible. When treated for infectious enteritis with paramomycin, cats have developed acute renal failure, deafness and cataracts (Gookin et al., 1999). Total deafness was described in a dog after administration of 500 mg SC for 5 days (Fowler, 1968). In cattle, parenterally administered neomycin causes nephrotoxicity and deafness, which may be enhanced by dehydration. In pigs, transient posterior paresis and apnea immediately after injection have resulted from neuromuscular blockade. In horses, IM administration of 10 mg/kg caused renal tubular injury (enzymuria and cylindriuria) within four days of neomycin administration (Edwards et al., 1989).

Administration and Dosage

Neomycin is reserved for local treatment of infections, often combined with bacitracin and polymyxin B

("triple antibiotic") for broad-spectrum synergistic activity. Framycetin is also used in topical preparations. Neomycin is routinely incorporated into "over-the-counter" oral formulations for enteritis in animals. These formulations are largely ineffective due to widespread resistance in the Enterobacteriaceae. In some countries neomycin is used as a parenteral injection for food animals and horses. Because of its toxic potential, such usage is strongly discouraged. It is also incorporated into combination formulations for mastitis in dairy cows.

Clinical Applications

Neomycin is used for the local treatment of intestinal infections; wound, otic, and skin infections; and mastitis. Its relatively broad-spectrum of activity and bactericidal effect made the drug popular in some countries for parenteral use in farm animals as an inexpensive "alternative" to gentamicin; safer, considerably less toxic, and more efficacious alternate drugs are now readily available.

Cattle, Sheep and Goats

Neomycin has been used in the oral treatment of enteric infections in ruminants, though resistance increasingly limits its effectiveness (Constable, 2004). Shull and Frederick (1978) found that routine addition of neomycin to milk powder for neonatal calves increased the frequency of diarrhea, possibly through a suppressive effect on normal intestinal microflora or through an irritant effect on the mucosa. Neomycin is absorbed after oral administration (approximately 3%) and may lead to kidney residues in cattle, especially veal calves with enteritis (Persoli et al., 1994; Shaikh B, et al., 1995). Routine intrauterine administration of neomycin boluses to postparturient cattle significantly increased the number of services per conception compared to controls (Fuquay et al., 1975). Neomycin is commonly incorporated into intramammary formulations for mastitis. But the use of a penicillin G-neomycin combination did not increase the efficacy of the treatment over that achieved by using penicillin G alone in bovine clinical mastitis caused by penicillin-susceptible, Gram-positive bacteria (Taponen et al., 2003).

Paramomycin is available in some countries for parenteral use for the treatment of bovine respiratory disease. Paramomycin may be an effective treatment for acute cryptosporidiosis caused by *Cryptosporidium*

parvum (Rehg, 1994; Mancassola et al., 1995) but may be less effective than azithromycin for this purpose.

Swine

Neomycin is used in the oral treatment of *E. coli* diarrhea, although resistance increasingly limits its use.

Horses

Neomycin may be used in the local treatment of infections caused by susceptible bacteria. Evidence of nephrotoxicity was observed in adult horses within four days of parenteral administration (Edwards et al., 1989). Oral administration is suggested for selective intestinal decontamination in horses with hepatic encephalopathy.

Dogs and Cats

Neomycin is used in combination formulations for the local treatment of infections in dogs and cats, such as otitis externa, bacterial keratitis and anal sac infections. Paramomycin has been used in the treatment of cryptosporidiosis in cats (Barr et al., 1994) and leishmaniasis in dogs (Oliva et al., 1998).

Poultry

Neomycin is sometimes administered orally to chickens and turkeys in the treatment of *Salmonella* infections.

Bibliography

- Barr SC, et al. 1994. Use of paramomycin for treatment of cryptosporidiosis in a cat. *J Am Vet Med Assoc* 205(12): 1742.
- Constable PD. 2004. Antimicrobial use in the treatment of calf diarrhea. *J Vet Intern Med* 18:8.
- Edwards DJ, et al. 1989. The nephrotoxic potential of neomycin in the horse. *Equine Vet J* 21:206.
- Fowler NG. 1968. The ototoxicity of neomycin in the dog. *Vet Rec* 82:267.
- Fuquay JW, et al. 1975. Routine postpartum treatment of dairy cattle with intrauterine neomycin sulfate boluses. *J Anim Sci* 58:1367.
- Gookin JL, et al. 1999. Acute renal failure in four cats treated with paramomycin. *J Am Vet Med Assoc* 215:1821.
- Mancassola R, et al. 1995. Chemoprophylaxis of *Cryptosporidium parvum* infection with paramomycin in kids and immunological study. *Antimicrob Agents Chemother* 39:75.
- Persoli WM, et al. 1994. Disposition and bioavailability of neomycin in Holstein calves. *J Vet Pharm Ther* 17:5.

- Oliva G, et al. 1998. Comparative efficacy of meglumine antimoniate and aminosidine sulphate, alone or in combination, in canine leishmaniasis. *Ann Trop Med Parasitol* 92(2):165.
- Rehg JE. 1994. A comparison of anticryptosporidial activity of paramomomycin with that of other aminoglycosides and azithromycin in immunosuppressed rats. *J Infect Dis* 170:934.
- Shaikh B, et al., 1995. Neomycin residues in kidneys of orally dosed non-ruminating calves determined by high-performance liquid chromatographic and microbiological assay methods. *J Vet Pharmacol Ther* 18:150.
- Shull JJ, Frederick HM. 1978. Adverse effect of oral antibacterial prophylaxis and therapy on incidence of neonatal calf diarrhea. *Vet Med Small Anim Clin* 73:924.
- Sparks TA, et al. 1994. Antimicrobial effect of combinations of EDTA-Tris and amikacin or neomycin on the microorganisms associated with otitis externa in dogs. *Vet Res Commun* 18:241.
- Taponen S, et al. 2003. Efficacy of intramammary treatment with procaine penicillin G vs. procaine penicillin G plus neomycin in bovine clinical mastitis caused by penicillin-susceptible, Gram-positive bacteria—a double blind field study. *J Vet Pharmacol Ther* 26:193.

Kanamycin Group

The kanamycin group contains the kanamycins and semisynthetic derivatives such as amikacin, the nebramycins such as tobramycin and apramycin, and gentamicin, netilmicin, and sisomicin.

Kanamycin

Antimicrobial Activity

Kanamycin (Figure 13.2) has similar activity to neomycin. It is active against many species of mycobacteria and mycoplasma but is inactive against *Pseudomonas aeruginosa* and anaerobes (Table 13.1). Bacteria with an MIC ≤ 16 $\mu\text{g/ml}$ are regarded as susceptible, of 32 $\mu\text{g/ml}$ as intermediate, and of ≥ 64 $\mu\text{g/ml}$ as resistant.

Resistance

Plasmid-mediated resistance can occur through a variety of enzymes (Table 13.2). Chromosomal resistance develops slowly but is far less important. Cross-resistance occurs with neomycin and one-way cross-resistance with streptomycin. Acquired resistance of *Escherichia coli* and other Gram-negative rods occurs frequently.

Toxicity and Adverse Effects

Kanamycin has a larger therapeutic index than neomycin but is less toxic on a weight basis (Table 13.4).

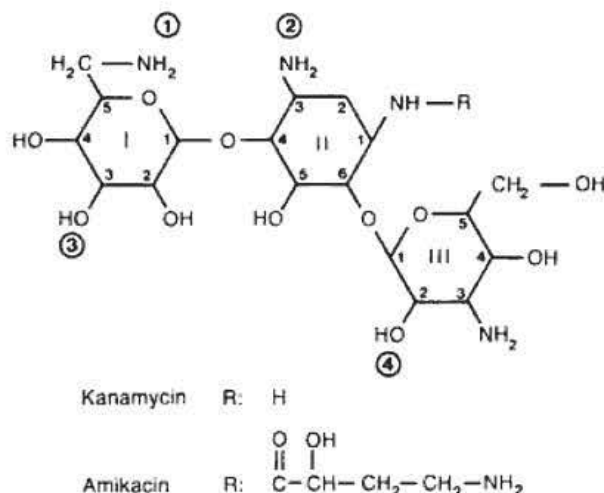


Figure 13.2. Structural formulas of kanamycin and amikacin.

Although excessively high doses are toxic to dogs and cats, cats given 100 mg/kg daily SC over 30 days showed no ill effects, and neither did dogs given the same dose over 9 months (Yeary, 1975).

Clinical Applications

Kanamycin has been largely replaced for parenteral administration by more active aminoglycosides. For local applications it offers no advantage over neomycin. Kanamycin is only available in the United States as an oral product for bacterial enteritis in dogs in combination with antidiarrheals, but some injectable formulations are still available in Europe.

Bibliography

- Yeary RA. 1975. Systemic toxic effects of chemotherapeutic agents in domestic animals. *Vet Clin North Am* 5:511.

Amikacin

Amikacin is a chemical modification of kanamycin (Figure 13.2), with greater activity than kanamycin on a weight basis, but with similar activity on a weight basis to gentamicin or tobramycin (Table 13.5). The remarkable property of amikacin is its resistance to most of the enzymes that inactivate the other aminoglycosides, including gentamicin and tobramycin (Table 13.2). This property is particularly important in the treatment of *P. aeruginosa* infections.

Table 13.5. Activity (MIC₉₀) of tobramycin, amikacin, and apramycin (µg/ml) against selected bacteria.

Organism	Tobramycin	Amikacin	Apramycin
Gram-positive aerobes			
<i>Nocardia</i> spp.	>32	2	
<i>Rhodococcus equi</i>	1	≤0.25	
<i>Staphylococcus aureus</i>	8	4	1
<i>Streptococcus pyogenes</i>	64	256	32
Gram-negative aerobes			
<i>Actinobacillus</i> spp.	2	8	16
<i>Bordetella bronchiseptica</i>	2	8	16
<i>Campylobacter jejuni</i>	2	2	
<i>Escherichia coli</i>	0.5	2	8
<i>Klebsiella pneumoniae</i>	8	4	4
<i>Pasteurella multocida</i>	2	8	16
<i>Proteus</i> spp.	1	4	8
<i>Pseudomonas aeruginosa</i>	8	16	16
<i>Salmonella</i> spp.	2	4	8

Antimicrobial Activity

Susceptible bacteria (MIC ≤16 µg/ml) are the Enterobacteriaceae including gentamicin-resistant *Enterobacter* spp., *E. coli*, *Klebsiella* spp., *Proteus* spp., and *Serratia* spp. Among Gram-positive bacteria, *Nocardia* spp. and *S. aureus* are sensitive (Table 13.5).

Resistant bacteria (MIC ≥64 µg/ml) include anaerobes, streptococci, and some *Pseudomonas* spp.

Antimicrobial Resistance

Emergence of resistance has been uncommon compared to gentamicin and other newer aminoglycosides (Table 13.2), but hospital-associated plasmid-mediated resistance in Gram-negative bacteria has been described (Orsini et al., 1989).

Drug Interactions

Amikacin is synergistic with beta-lactams, for example, with azlocillin or ticarcillin against *P. aeruginosa*.

Pharmacokinetic Properties

Amikacin's properties are typical of aminoglycosides. Reported volumes of distribution range from 0.15 to 0.3 L/kg and plasma elimination half-lives range from 1 to 2 hours in adult animals. Protein binding is low. Elimination half-lives are prolonged in neonates, especially if they are septic or hypoxic (Green and Conlon,

1993; Wichtel et al., 1992). Bioavailability from IM or SC injection is high (90–100%). Amikacin is distributed into peritoneal fluid and synovial fluid in the horse.

Toxicity and Adverse Effects

Amikacin may be slightly less nephrotoxic and ototoxic than kanamycin, with the frequency of these effects in humans being low. In animals with normal renal function, amikacin administered at recommended doses for 2 to 3 weeks is unlikely to produce toxic effects. Monitoring of renal function during treatment is recommended. Concerns that decreased glomerular filtration in neonatal foals might lead to a need to reduce dosage to prevent nephrotoxicity were reported to be unfounded by Adland-Davenport et al. (1990), since renal clearance was greater in foals than was observed in adults. Dosage should be adjusted in cases of pre-existing renal impairment, preferably guided by therapeutic drug monitoring (see discussion under Gentamicin).

Administration and Dosage

Suggested drug dosages are shown in Table 13.4. Amikacin is labeled for intrauterine use in horses and IM or SC use in dogs. It is frequently administered IV, SC, IM, by intra-articular injection, or by localized venous or intraosseous perfusion in many species.

Clinical Applications

Amikacin is a broad-spectrum, bactericidal drug useful for severe illness in animals, for example, in Gram-negative septicemia caused by gentamicin-resistant organisms, as might be encountered in a veterinary hospital. In human medicine, it is often combined with anti-pseudomonal penicillins in the treatment of *P. aeruginosa* infections in neutropenic patients.

Horses

Amikacin has been licensed for use in the United States and Canada for the treatment of bacterial endometritis of mares and should be reserved for *P. aeruginosa* and *K. pneumoniae* infections, as activity against *Streptococcus zooepidemicus* is poor. The drug had no adverse effects on conception in horses when used in a pH-buffered semen extender at 100 µg/ml (Blue and Oriol 1982). An intrauterine infusion of 2 g in 200 ml saline for 3 days gave highest overall cure rates against experimental *Klebsiella* metritis in mares

and 94–100% cure rates in *Pseudomonas* or *Klebsiella* metritis under field conditions (Gingerich et al., 1983). Others have successfully used the same treatment for 5 days (Brook, 1982). Pharmacokinetic studies support the use of 2 g intrauterine infusions once daily rather than IM treatment (Orsini et al., 1996).

Amikacin is used in neonatal foals in the treatment of septicemia or pneumonia. Magdesian and others (2004) found that a once-daily dose of 21 mg/kg in foals did not cause nephrotoxicity and suggested that once-daily dosing might be more efficacious than divided daily dosing, for reasons discussed earlier. As efficacy correlates to the C_{max}:MIC ratio, an initial dosage of 25 mg/kg q 24 hours is suggested for foals to achieve peak concentrations of >40 µg/ml (Bucki et al., 2004).

Amikacin is also used in the treatment of musculoskeletal infections caused by *Staphylococcus* spp. and Gram-negative bacteria. Due to the expense of systemic therapy, it is often administered intra-articularly, or by regional intravenous or intraosseous perfusion to the distal limbs. Such local administration results in high amikacin concentrations in joints and tendon sheaths, and avoids systemic toxicity (Butt et al., 2001). When performing intra-articular injections with corticosteroids or chondroprotective drugs (e.g., hyaluronate), because of the catastrophic consequences of sepsis, a small amount of amikacin is frequently added to the therapy (Dabareiner et al., 2003).

Dogs and Cats

Amikacin has been approved for parenteral use in dogs in the United States and Canada. It is used in cats as well. Indications include serious Gram-negative infections (cystitis, skin or soft tissue infections) caused by otherwise resistant Enterobacteriaceae or *P. aeruginosa*, for which alternate drugs are not available or appropriate.

Bibliography

- Adland-Davenport P, et al. 1990. Pharmacokinetics of amikacin in critically ill neonatal foals treated for presumed or confirmed sepsis. *Equine Vet J* 22:18.
 Blue MG, Oriol JG. 1982. Conception in mares following intrauterine therapy with amikacin. *J Eq Vet Sci* 2:200.
 Brook D. 1982. Using amikacin sulfate to treat genital infections in mares. *Vet Med Small Anim Clin* 77:434.

- Bucki EP, et al. 2004. Pharmacokinetics of once-daily amikacin in healthy foals and therapeutic drug monitoring in hospitalized equine neonates. *J Vet Intern Med* 18:728.
 Dabareiner RM, et al. 2003. Injection of corticosteroids, hyaluronate, and amikacin into the navicular bursa in horses with signs of navicular area pain unresponsive to other treatments: 25 cases (1999–2002). *J Am Vet Med Assoc* 223:1469.
 Green SL, Conlon PD. 1993. Clinical pharmacokinetics of amikacin in hypoxic premature foals. *Equine Vet J* 25:276.
 Jernigan AD, et al. 1988. Pharmacokinetics of amikacin in cats. *Am J Vet Res* 49:355.
 Magdesian KG, et al. 2004. Pharmacokinetics and nephrotoxicity of high dose amikacin administered at extended intervals to neonatal foals. *Am J Vet Res* 65:473.
 Orsini JA, et al. 1989. Resistance to gentamicin and amikacin of Gram-negative organisms isolated from horses. *Am J Vet Res* 50:923.
 Orsini JA, et al. 1996. Tissue and serum concentrations of amikacin after intramuscular and intrauterine administration to mares in estrus. *Can Vet J* 37:157.
 Wichtel MG, et al. 1992. Relation between pharmacokinetics of amikacin sulfate and sepsis score in clinically normal and hospitalized neonatal foals. *J Am Vet Med Assoc* 200:1339.

Apramycin

Apramycin, like tobramycin, is a nebramycin isolated from the fermentation of *Streptomyces tenebrans*. It has not been developed for clinical use in humans but has been used in the oral treatment of Gram-negative bacterial enteritis of farm animals.

Apramycin is active against *S. aureus*, many Gram-negative bacteria, and some *Mycoplasma* spp. (Table 13.5). Additional studies are required to define its spectrum of activity. Bacteria with an MIC ≤16 µg/ml are regarded as susceptible.

The unique chemical structure of apramycin resists most of the R-plasmid-mediated degradative enzymes. Resistance is thus rare among Gram-negative bacteria, so that many pathogenic *E. coli* and *Salmonella* isolated from animals are susceptible. Resistance develops only through one enzyme pathway, aminoglycoside 3-N-acetyltransferase IV. Such unique resistance has been used as a marker in studies which support the view that resistance in human nonclinical and clinical isolates of bacteria can spread from use of apramycin in animals (Chaslus-Dancla et al., 1991; Hunter et al., 1994; Johnson et al., 1994). Reports from France by Chaslus-Dancla et al. (1986), however, described multiple aminoglycoside resistance in bovine *Salmonella*

typhimurium isolates, because of the presence of resistance plasmids encoding AAC(3)IV. Chromosomal cross-resistance does not occur with other aminoglycosides.

In calves, administration of 20 or 40 mg/kg orally or 20 mg/kg IM for 5 days significantly reduced losses from experimental salmonellosis (Espinasse et al., 1981). In veal calves with naturally occurring salmonellosis, 40 mg/kg orally was marginally more effective than 20 mg/kg (Bayle et al., 1980). The pharmacokinetic studies of Ziv et al. (1985) led them to suggest a dose of 20 mg/kg IM every 12 or 24 hours for the treatment of *Salmonella* or *E. coli* infections, respectively.

In swine, apramycin is highly effective in prophylaxis and treatment of colibacillosis (Andreotis et al., 1980). The drug is incorporated into water so that pigs consume sufficient amounts to obtain 12.5 mg/kg daily for seven days. Efficacy has been reported against naturally acquired *E. coli* infections in broilers (Cracknell et al., 1986). Enteritis significantly increases oral absorption in chickens which may be problematic for tissue residues (Thomson et al., 1992). Tissue residues are a problem typical of aminoglycosides in general.

Bibliography

- Andreotis JS, et al. 1980. An evaluation of apramycin as an in-feed medication for the treatment of postweaning colibacillosis in pigs. *Vet Res Comm* 4:131.
- Bayle R, et al. 1980. L'apramycine. Un nouvel antibiotique de la famille aminosides. *Bull Mens Soc Vet Prat Fr* 64:371.
- Chaslus-Dancla E, et al. 1986. Emergence of aminoglycoside 3-N-acetyltransferase IV on *Escherichia coli* and *Salmonella typhimurium* isolated from animals in France. *Antimicrob Agents Chemother* 29:239.
- Chaslus-Dancla E, et al. 1991. High genetic homology between plasmids of human and animal origins conferring resistance to the aminoglycosides gentamicin and apramycin. *Antimicrob Agents Chemother* 35:590.
- Cracknell VC, et al. 1986. An evaluation of apramycin soluble powder for the treatment of naturally acquired *Escherichia coli* infections in broilers. *J Vet Pharmacol Ther* 9(3):273.
- Espinasse J, et al. 1981. Reproduction experimentale de la salmonellose chez le veau-traitement par l'apramycine. *Rec Med Vet* 157:571.
- Hunter JEB, et al. 1992. Apramycin resistance plasmids in *Escherichia coli*: possible transfer to *Salmonella typhimurium* in calves. *Epidemiol Infect* 108:271.
- Hunter JEB, et al. 1994. Apramycin-resistant *Escherichia coli* isolated from pigs and a stockman. *Epidemiol Infect* 112:473.
- Johnson AP, et al. 1994. Gentamicin resistance in clinical isolates of *Escherichia coli* encoded by genes of veterinary origin. *J Med Microbiol* 40:221.
- Thomson TD, et al. 1992. Effects of intestinal pathology due to coccidial infection on the oral absorption of apramycin in 4-week-old broiler chickens. *Acta Vet Scand Suppl* 87:275.
- Ziv G, et al. 1985. Clinical pharmacology of apramycin in calves. *J Vet Pharm Ther* 8:95.

Gentamicin

Gentamicin is one of the fermentation products of *Micromonospora purpurea*; because it is not a *Streptomyces* product, it is spelled "gentamicin," not "gentamycin".

Antimicrobial Activity

Gentamicin is one of the most active aminoglycosides (Table 13.1). The drug is active against most Gram-negative aerobic rods including many *P. aeruginosa*, against some Gram-positive bacteria, and against *Mycoplasma* spp. It is usually more active against streptococci than amikacin. Gentamicin has little activity against mycobacteria or *Nocardia*, and none against anaerobic bacteria or against aerobic bacteria under anaerobic conditions. Like all aminoglycosides, it is a bactericidal, concentration-dependent killer and penetrates phagocytic cells poorly. There is widespread susceptibility among veterinary pathogenic bacteria although resistance is sometimes a problem in veterinary hospital settings (Peyrou et al., 2003; Sanchez et al., 2002). In human hospitals, there have been explosive outbreaks of nosocomial infection caused by gentamicin-resistant bacteria of many species.

Susceptible bacteria (MIC \leq 4 μ g/ml) are most Enterobacteriaceae including *Enterobacter* spp., *E. coli*, *Klebsiella* spp., *Proteus* spp., *Serratia* spp., *Yersinia* spp., *Brucella* spp., *Campylobacter* spp., *Haemophilus* spp., and *Pasteurella* spp. Most strains of *Pseudomonas aeruginosa* are susceptible. Among Gram-positive bacteria, *S. aureus* are susceptible but susceptibility of streptococci and many other Gram-positive aerobes can be variable. *Prototheca zopfii* are generally susceptible. *Rhodococcus equi* is susceptible in vitro, but clinical efficacy is poor due to poor penetration and activity in abscesses.

Resistant bacteria ($MIC \geq 16 \mu\text{g/ml}$) include many Gram-positive aerobes, some *Pseudomonas* spp., and anaerobes. Strains of gentamicin-resistant *P. aeruginosa* are commonly susceptible to amikacin or tobramycin (Table 13.3).

Pharmacokinetic Properties

Like amikacin, reported values of distribution for gentamicin range from 0.15 to 0.3 L/kg, and plasma elimination half-lives range from 1 to 2 hours in adult animals. Protein binding is low. The larger volume of distribution in neonates means that dosage in these animals should be higher than in adults but dosage intervals need to be extended (see Clinical Applications).

Drug Interactions

Gentamicin is commonly synergistic with beta-lactam antibiotics against a wide variety of Gram-negative rods, including *P. aeruginosa* (azlocillin, piperacillin), as long as the bacteria are susceptible to the beta-lactam and gentamicin. It is commonly synergistic with beta-lactam antibiotics against Gram-positive bacteria such as *Rhodococcus equi* and *Listeria monocytogenes*. Gentamicin is commonly synergistic with trimethoprim-sulfonamide combinations against *E. coli* and *K. pneumoniae*. Antagonism may occur with chloramphenicol, tetracycline, and erythromycin.

Carbenicillin, a drug with which gentamicin previously was used to treat serious *Pseudomonas* infections in humans, is incompatible with gentamicin in vitro, as are all beta-lactams. Penicillins and gentamicin should not be mixed in vitro and when both drugs are administered through the same intravenous line, care must be taken to flush thoroughly between drugs.

Halothane anesthesia causes significant changes in the pharmacokinetics of gentamicin in horses; total body clearance and volume of distribution decrease while half-life of elimination increases (Smith et al., 1988). A longer gentamicin dosing interval after anesthesia may help correct for the changes, but serious consideration should be given to choice of another antimicrobial. In horses, concurrent administration of phenylbutazone with gentamicin decreases the elimination half-life of gentamicin by 23% and decreases the volume of distribution by 26%, while the pharmacokinetics of phenylbutazone are not affected (Whittem et al., 1996).

Toxicity and Adverse Effects

Gentamicin has the usual aminoglycoside toxic effects of neuromuscular blockade, which is exacerbated by anesthetics, and of minor cardiovascular depressive effects; the drug should not be given rapidly IV. Gentamicin is potentially ototoxic, but the major toxic effect is nephrotoxicosis, which limits prolonged use. High trough concentrations are associated with nephrotoxicosis due to accumulation in renal tubular epithelial cells. Because of the nephrotoxic potential of gentamicin, it is best reserved for severe infections. Ideally, serum drug concentrations should be monitored in treated animals. Otherwise, careful monitoring of renal function is required.

Subclinical renal damage, which occurs with most gentamicin therapeutic regimens, is generally reversible. This is not of clinical significance if there is no pre-existing renal disease. Risk factors enhancing nephrotoxicity include immaturity or old age, acidosis, concurrent use of diuretics such as furosemide, daily and total dose, fever, dehydration, previous aminoglycoside treatment, concurrent treatment with amphotericin B and possibly phenylbutazone and, in the dog, pyometra. Fever decreases clearance and volume of distribution, thus increasing plasma gentamicin concentrations.

Currently, high-dose, once-daily gentamicin therapy is recommended to maximize antimicrobial efficacy and minimize nephrotoxicity. Monitoring peak and trough serum concentrations to detect changes in the elimination half-life is the most proactive way to detect the onset of nephrotoxicity, but may be difficult to do in a clinical setting. The next best indicator is an increase in urine gamma glutamyl transferase (GGT) and an increase in the urine GGT:urine creatinine (Cr) ratio. Elevations in serum urea nitrogen and Cr confirm nephrotoxicity, but are not seen for seven days after significant renal damage has occurred. Elimination half-lives of 24 to 45 hours have been reported in horses with renal toxicity, further prolonging the toxic exposure to the drug (Sweeney et al., 1988). While peritoneal dialysis is useful in lowering creatinine and serum urea nitrogen, it may not be effective in significantly increasing the elimination of the accumulating aminoglycoside. Nomograms based on age and renal function are used in calculating gentamicin dosage in human patients but are not available in veterinary medicine. Recent population pharmacokinetic studies of gentamicin in horses showed that a consid-

Table 13.6. Applications of gentamicin to clinical infections in animals.

Species	Primary Application	Comments
Horses	Gram-negative septicemia in foals, pleuropneumonia and surgical prophylaxis for colic surgery in adult horses. Metritis in mares. Infectious keratitis. Severe Gram-negative infections.	Nephrotoxicity limits use.
Dogs, cats	Gram-negative sepsis. Infectious keratitis. Otitis externa.	
Cattle, sheep, goats	Not recommended due to prolonged kidney residues.	
Pigs	Neonatal colibacillosis.	
Poultry	Gram-negative septicemia in poults and chicks.	

erable proportion of the individual variability recognized in gentamicin disposition could be explained by differences in body weight and serum creatinine (Martin-Jimenez et al., 1998), and that such data can be used to estimate dosage for single daily dosing. Renal damage in dogs administered the recommended dosage is usually mild or moderate and reversible (Albarellos et al., 2004).

Nephrotoxicity can be decreased by feeding the patient a high protein diet/high calcium diet such as alfalfa to large animals and diets higher than 25% protein to small animals, as protein and calcium cations compete with gentamicin cations for binding to renal tubular epithelial cells (Behrend et al., 1994; Schumacher et al., 1991). High dietary protein also increases glomerular filtration rate and renal blood flow, thereby reducing gentamicin accumulation. The sparing effect of feeding may be related to the competitive inhibition by protein at the proximal tubule or the nephrotoxic-sparing effect of calcium (Brashier et al., 1998).

Cats are susceptible to gentamicin toxicosis, which is manifested as loss in vestibular function, which precedes nephrotoxicity. Therapeutic dosage is usually safe in cats treated for reasonable periods (5 days) (Waitz et al., 1971; Hardy et al., 1985). Monitoring of renal function or therapeutic drug monitoring is advised in seriously ill cats, for which the drug should be reserved.

Administration and Dosage

Administration and dosages for major-use species are shown in Table 13.4. Gentamicin is labeled for intrauterine use in horses, IM or PO use in piglets, SC use in poultry, and IM or SC use in dogs. It is frequently administered IV, SC, IM, by intra-articular in-

jection, and by intravenous or intraosseous perfusion. It is used in many other species, as well.

Clinical Applications

Clinical applications of gentamicin are shown in Table 13.6. Gentamicin is a bactericidal drug active against aerobic bacteria, especially Gram-negative bacteria, and is particularly useful for its activity against Enterobacteriaceae and *P. aeruginosa*. It is a drug of choice in the treatment of severe sepsis caused by Gram-negative aerobic rods, but fluoroquinolones have a similar spectrum of activity with better tissue distribution and safety profiles. It is often combined with a beta-lactam drug for synergistic activity. Combination treatment of severe sepsis with gentamicin, ampicillin, and metronidazole gives a broad-spectrum antibacterial effect.

In the absence of factors predisposing to gentamicin nephrotoxicosis, treatment with high-dose, once-daily therapy for 5–7 days is unlikely to cause significant renal damage. Because of its persistence in the kidneys following treatment, it is not recommended for systemic use in food animals in the United States or Canada. If used, the Food Animal Residue Avoidance Databank recommends an 18-month withdrawal time.

Local application in the case of local infections (otitis, keratitis, metritis) usually overcomes the problem of toxicity. Nephrotoxicosis in a cat associated with excessive infusion of gentamicin into an abscess was described (Mealey and Boothe, 1994). Gentamicin-impregnated polymethyl methacrylate beads can be used in the treatment of bone and joint infections, since they result in a slow release of gentamicin at the site of infection and thus avoid problems of nephrotoxicity.

Cattle, Sheep, and Goats

Gentamicin has had limited use in these species because of cost and prolonged tissue residues. It has been used extra-label in the treatment of coliform mastitis in dairy cows. One well-conducted field study of cows suffering from coliform mastitis showed no beneficial effects of systemic administration of the drug (Jones and Ward, 1990). The benefit of intramammary application has been questioned (Erskine et al., 1991) and, experimentally, intramammary gentamicin had no beneficial effect on the course of *E. coli* mastitis in cows (Erskine et al., 1992). Such intramammary use may also cause violative kidney residues (Persoli et al., 1995; Sweeney et al., 1996).

Swine

Gentamicin is used to treat neonatal colibacillosis from day 1 to day 3 of age with either a single IM injection or an oral dose of 5 mg. If multiple doses are given or if administered to older piglets, a significantly increased withdrawal time should be followed.

Horses

Gentamicin is widely used for the treatment of serious bacterial infections in horses because of its relatively broad activity against Gram-negative aerobes, its relatively low cost, and the wide prevalence of susceptible bacteria. Gentamicin is frequently administered with a beta-lactam antimicrobial to horses undergoing colic surgery (Traub-Dargatz et al., 2002). Endotoxemia increases the elimination half-life of gentamicin in these horses (Sweeney et al., 1992; van der Harst et al., 2005) but pharmacokinetics are not altered by fluid administration (Jones et al., 1998) or peritoneal lavage (Easter et al., 1997). The risk of nephrotoxicity can be reduced by providing a diet high in protein and calcium, such as alfalfa hay (Schumacher et al., 1991).

In foals, gentamicin is often used in the treatment of Gram-negative septicemia, but because of its poor penetration of the blood-brain barrier it is ineffective in the treatment of meningitis. The drug should not be used for more than 5–7 days without monitoring renal toxicity and trough serum concentrations (Wichtel et al., 1999).

Gentamicin is used in the treatment of infectious metritis in mares caused by susceptible *S. zooepidemicus*, *K. pneumoniae* or *P. aeruginosa*. It has given excellent results when infused into the uterus during estrus on a daily basis for 3 to 5 days (250 ml physiologic

saline, 10 mg/ml) (Houdeshell and Hennessey, 1972). Gentamicin should not be used routinely at or before service or insemination to avoid promoting resistance and destroying normal vaginal microflora. Stallions with *Klebsiella* or *Pseudomonas* infections of the genital tract have been successfully treated with 4.4 mg/kg twice daily IM or IV (Hamm, 1978).

Gentamicin is often a first-line choice for bacterial ulcerative keratitis. Susceptibility testing should be done; however, as increasing resistance to gentamicin has been observed in corneal pathogens and ineffective therapy may be catastrophic (Sauer et al., 2003).

Gentamicin is administered intra-articularly in the treatment of septic arthritis in horses, since concentrations in synovial fluid achieved by this route exceed those achieved by parenteral administration by up to 100 times, thus exceeding the MIC of susceptible pathogens for 24 hours (Lescun et al., 2002; Meijer et al., 2000). Intraosseous or intravenous regional perfusion also achieves high local concentrations for the treatment of septic arthritis or osteomyelitis (Werner et al., 2003; Mattson et al., 2004). Gentamicin-impregnated polymethyl methacrylate beads are also successfully used to treat septic arthritis (Booth et al., 2001; Farnsworth et al., 2001).

Dogs and Cats

The widespread susceptibility of common bacterial pathogens of dogs and cats has made gentamicin a popular drug in small animal practice, where it has been used with excellent results in infections of the urinary tract, respiratory tract, skin and soft tissue, eyes (superficial infections), and gastrointestinal tract. Gentamicin-impregnated polymethyl methacrylate beads can be used for local therapy of musculoskeletal infections. Gentamicin's activity against *Staphylococcus intermedius* and *P. aeruginosa* has made it especially useful for topical treatment of canine otitis externa. But unless predisposing factors are corrected, *P. aeruginosa* often becomes resistant in chronic cases (Martin Barrasa et al., 2000; Seol et al., 2002). When applied topically to clinically normal dogs with intact or ruptured tympanic membranes, gentamicin does not cause detectable cochlear or vestibular damage (Strain et al., 1995). Apart from local application, it should be reserved for serious infections for which other drugs are not suitable. Nevertheless, as discussed earlier, the nephrotoxic potential of gentamicin should be kept in perspective. Administration for 5–7 days in

animals not predisposed to gentamicin nephrotoxicosis is unlikely to produce significant renal damage. The risk can be reduced by feeding a high-protein diet (Behrend et al., 1994). Therapeutic drug monitoring should be done in animals with factors predisposing to gentamicin nephrotoxicosis.

Poultry

Gentamicin is administered SC to 1- to 3-day old poults and 1-day old chicks in the prevention and treatment of *E. coli*, *P. aeruginosa*, *Arizona paracolon* and *Salmonella* infections.

Camelids

Gentamicin is used in camelids for treatment of Gram-negative infections. Camelids appear susceptible to gentamicin-induced nephrotoxicity (Hutchison et al., 1993). A pharmacokinetic study in normal adult llamas demonstrated high peak concentrations and prolonged elimination times, suggesting that gentamicin should be administered at lower doses with long dosing intervals (Dowling et al., 1996).

Bibliography

- Albarellos G, et al. 2004. Multiple once-daily dose pharmacokinetics and renal safety of gentamicin in dogs. *J Vet Pharmacol Ther* 27:21.
- Behrend EN, et al. 1994. Effects of dietary protein conditioning on gentamicin pharmacokinetics in dogs. *J Vet Pharmacol Ther* 17:259.
- Booth TM, et al. 2001. Treatment of sepsis in the small tarsal joints of 11 horses with gentamicin-impregnated polymethyl methacrylate beads. *Vet Rec* 148:376.
- Brashier MK, et al. 1998. Effect of intravenous calcium administration on gentamicin-induced nephrotoxicosis in ponies. *Am J Vet Res* 59:1055.
- Brown SA, Garry FB. 1988. Comparison of serum and renal gentamicin concentrations with fractional urinary excretion tests as indicators of nephrotoxicity. *J Vet Pharmacol Ther* 11:330.
- Dowling PM, et al. 1996. Pharmacokinetics of gentamicin in llamas. *J Vet Pharmacol Ther* 19:161-163.
- Easter JL, et al. 1997. Effects of postoperative peritoneal lavage on pharmacokinetics of gentamicin in horses after celiotomy. *Am J Vet Res* 58:1166.
- Erskine RJ, et al. 1991. Theory, use, and realities of efficacy and food safety of antimicrobial treatment of acute coliform mastitis. *J Am Vet Med Assoc* 198:980.
- Erskine RJ, et al. 1992. Intramammary gentamicin as a therapy for experimental *Escherichia coli* mastitis. *Am J Vet Res* 53:375.
- Farnsworth, KD et al. 2001. The effect of implanting gentamicin-impregnated polymethylmethacrylate beads in the tarsocrural joint of the horse. *Vet Surg* 30:126.
- Hamm DH. 1978. Gentamicin therapy of genital tract infections in stallions. *J Equine Med Surg* 2:243.
- Hardy ML, et al. 1985. The nephrotoxic potential of gentamicin in the cat: Enzymuria and alterations in urine concentrating capability. *J Vet Pharm Ther* 8:382.
- Houdeshell JW, Hennessey PW. 1972. Gentamicin in the treatment of equine metritis. *Vet Med Small Anim Clin* 67:1348.
- Hutchison JM, et al. 1993. Acute renal failure in the llama (*Lama glama*). *Cornell Vet* 83:39-46.
- Jones GF, Ward GE. 1990. Evaluation of systemic administration of gentamicin for treatment of coliform mastitis in cows. *J Am Vet Med Assoc* 197:731.
- Jones SL, et al. 1998. Pharmacokinetics of gentamicin in healthy adult horses during intravenous fluid administration. *J Vet Pharmacol Ther* 21:247.
- Lescun TB, et al. 2002. Effects of continuous intra-articular infusion of gentamicin on synovial membrane and articular cartilage in the tarsocrural joint of horses. *Am J Vet Res* 63:683.
- Magdesian KG, et al. 1998. Pharmacokinetics of a high dose of gentamicin administered intravenously or intramuscularly in horses. *J Am Vet Med Assoc* 213:1007.
- Martin Barrasa JL, et al. 2000. Antibacterial susceptibility patterns of *Pseudomonas* strains isolated from chronic canine otitis externa. *J Vet Med B Infect Dis Vet Public Health* 47:191.
- Martin-Jimenez T, et al. 1998. Population pharmacokinetics of gentamicin in horses. *Am J Vet Res* 59:1589.
- Mattson S, et al. 2004. Intraosseous gentamicin perfusion of the distal metacarpus in standing horses. *Vet Surg* 33:180.
- Mealey KL, Boothe DM. 1994. Nephrotoxicosis associated with topical administration of gentamicin in a cat. *J Am vet Med Assoc* 204:1919.
- Meijer MC, et al. 2000. Clinical experiences of treating septic arthritis in the equine by repeated joint lavage: a series of 39 cases. *J Vet Med A Physiol Pathol Clin Med* 47:351.
- Pedersoli WM, et al. 1995. Depletion of gentamicin in the milk of Holstein cows after single and repeated intramammary and parenteral treatments. *J Vet Pharm Ther* 18:457.
- Peyrou, M., R. Higgins, et al. 2003. Evolution of bacterial resistance to certain antibacterial agents in horses in a veterinary hospital. *Can Vet J* 44: 978-81.
- Rivers BJ, et al. 1996. Evaluation of urine gamma-glutamyl transpeptidase-to-creatinine ratio as a diagnostic tool in an experimental model of aminoglycoside-induced acute renal failure in the dog. *J Am Anim Hosp Assoc* 32:323.
- Riviere JE, et al. 1982. Gentamicin toxic nephropathy in horses with disseminated bacterial infection. *J Am Vet Med Assoc* 180:648.
- Sanchez S, et al. 2002. Characterization of multidrug-resistant *Escherichia coli* isolates associated with nosocomial infections in dogs. *J Clin Microbiol* 40:3586.
- Sauer P, et al. 2003. Changes in antibiotic resistance in equine bacterial ulcerative keratitis (1991-2000): 65 horses. *Vet Ophthalmol* 6:309.

- Schumacher J, et al. 1991. Effect of diet on gentamicin-induced nephrotoxicosis in horses. *Am J Vet Res* 52:1274.
- Seol B, et al. 2002. In vitro antimicrobial susceptibility of 183 *Pseudomonas aeruginosa* strains isolated from dogs to selected antipseudomonal agents. *J Vet Med B Infect Dis Vet Public Health* 49(4):188.
- Short CR, et al. 1986. The nephrotoxic potential of gentamicin in the cat: A pharmacokinetic and histopathologic investigation. *J Vet Pharm Ther* 9:325.
- Smith CM, et al. 1988. Effects of halothane anesthesia on the clearance of gentamicin sulfate in horses. *Am J Vet Res* 49:19.
- Stover SM, Pool RE. 1985. Effect of intra-articular gentamicin sulfate on normal equine synovial membrane. *Am J Vet Res* 46:2485.
- Strain GM, et al. 1995. Ototoxicity assessment of a gentamicin sulfate otic preparation in dogs. *Am J Vet Res* 56:532.
- Sweeney RW, et al. 1988. Kinetics of gentamicin elimination in two horses with acute renal failure. *Equine Vet J* 20:182.
- Sweeney RW, et al. 1992. Disposition of gentamicin administered intravenously to horses with sepsis. *J Am Vet Med Assoc* 200:503.
- Sweeney RW, et al. 1996. Systemic absorption of gentamicin following intramammary administration to cows with mastitis. *J Vet Pharm Ther* 19:155.
- Traub-Dargatz JL, et al. 2002. Survey of complications and antimicrobial use in equine patients at veterinary teaching hospitals that underwent surgery because of colic. *J Am Vet Med Assoc* 220:1359.
- Van der Harst MR, et al. 2005. Gentamicin nephrotoxicity: a comparison of in vitro findings with in vivo experiments in equines. *Vet Res Commun* 29:247.
- Van der Harst MR, et al. 2005. Influence of fluid therapy on gentamicin pharmacokinetics in colic horses. *Vet Res Commun* 29:141.
- Waitz JA, et al. 1971. Aspects of the chronic toxicity of gentamicin sulfate in cats. *J Infect Dis* 124(suppl):S125.
- Werner LA, et al. 2003. Bone gentamicin concentration after intra-articular injection or regional intravenous perfusion in the horse. *Vet Surg* 32:559.
- Whittem T, et al. 1996. Pharmacokinetic interactions between repeated dose phenylbutazone and gentamicin in the horse. *J Vet Pharmacol Ther* 19:454.
- Wichtel ME, et al. 1999. Pharmacologic considerations in the treatment of neonatal septicemia and its complications. *Vet Clin North Am Equine Pract* 15:725.
- Ziv G. 1981. Concentration and residues of gentamicin in the milk of dairy cows after intramammary infusion. *Bovine Pract* 16:30.

Spectinomycin

Spectinomycin (Figure 13.3) is a product of *Streptomyces spectabilis*. It is an aminocyclitol antibiotic that lacks most of the toxic effects of the aminoglycoside antibiotics but, unfortunately, is limited in application

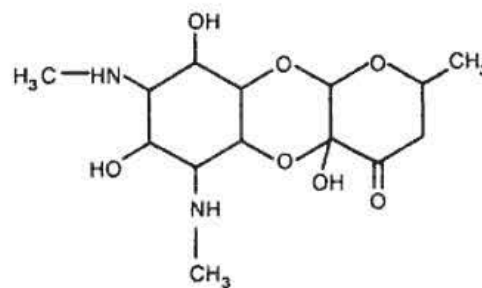


Figure 13.3. Structural formula of spectinomycin.

by the ready development of resistance. There are discrepancies between resistance to the drug in vitro and apparent efficacy in some cases clinically, which have not been explained. For example, Goren et al. (1988) observed high efficacy of orally administered spectinomycin or lincomycin-spectinomycin in treating experimentally induced *E. coli* infections in chickens, despite the absence of any antimicrobial activity in the serum of these chickens. To explain this discrepancy, they suggested that a metabolite or degradation product of the drug might reach the respiratory tract and interfere with bacterial attachment. This explanation is speculative since it has not been shown that the drug undergoes metabolism in any species. In humans, all of an administered dose is recovered in the urine within 48 hours after injection.

Antimicrobial Activity

Spectinomycin is a usually bacteriostatic, relatively broad-spectrum drug that can be bactericidal at concentrations four times MIC. It is not particularly active on a weight basis (Table 13.7). Bacteria are usually regarded as susceptible if their MIC is ≤ 20 $\mu\text{g/ml}$. Susceptibility among aerobic Gram-negative rods is unpredictable because of the presence of naturally resistant strains. *Mycoplasma* are susceptible but *P. aeruginosa* is resistant.

Resistance

Natural resistance to spectinomycin in many enteric bacteria is widespread. Chromosomal one-step mutation to high-level resistance develops readily in vivo and in vitro, in a manner similar to streptomycin resistance. Chromosomally resistant strains do not show cross-resistance with aminoglycosides. Plasmid-mediated resistance is uncommon. Vaillancourt et al. (1988) reported a marked drop (from 91% to 24%) of

in vitro susceptibility of *Actinobacillus pleuropneumoniae* isolated over a 5-year period, associated with the widespread use of the drug to treat pleuropneumonia in swine. They noted, however, discrepancies between in vitro resistance and apparent field efficacy. Susceptibility of Gram-negative pathogens involved in bovine respiratory disease is variable (Welsh et al., 2004). *Mycoplasma bovis* isolates can acquire resistance to spectinomycin (Francoz et al., 2005).

Drug Interactions

Combination with lincomycin may marginally enhance spectinomycin's activity against mycoplasma and *Lawsonia intracellularis*.

Toxicity and Adverse Effects

Spectinomycin seems to be relatively nontoxic in animals; it does not induce ototoxicity or nephrotoxicity but may, like the aminoglycosides, cause neuromuscular blockade. The apparent lack of reported toxic effects may reflect lack of long-term usage. Administration of lincomycin-spectinomycin oral preparations, by parenteral injection to cattle, has produced heavy losses associated with severe pulmonary edema. Similar problems have been noted with misuse of spectinomycin, and attributed to endotoxin contamination (Genetsky et al., 1994).

Pharmacokinetic Properties

Pharmacokinetic properties are similar to those of the aminoglycosides.

Administration and Dosage

Administration and dosages are shown in Table 13.4.

Clinical Applications

Spectinomycin has been largely abandoned in human medicine because of the rapid development of resistance and unpredictable antibiotic susceptibility. The drug is used in animals in the treatment of *Mycoplasma* infections, diseases caused by Enterobacteriaceae (*E. coli* diarrhea and septicemia), and respiratory disease caused by Gram-negative bacteria. The development of resistance in bacteria limits its long-term use. It is sometimes combined with lincomycin to give a broad-spectrum combination with activity against Gram-positive aerobic as well as anaerobic bacteria.

In cattle, spectinomycin is labeled for SC injection

Table 13.7. Activity (MIC₉₀) of spectinomycin (μg/ml) against selected bacteria and mycoplasma.

Organism	MIC ₉₀ (μg/ml)
Gram-positive aerobes	
<i>Rhodococcus equi</i>	8
<i>Staphylococcus aureus</i>	64
<i>Streptococcus pyogenes</i>	64
Gram-negative aerobes	
<i>Actinobacillus pleuropneumoniae</i>	32
<i>Bordetella avium</i>	>128
<i>B. bronchiseptica</i>	>256
<i>Brucella canis</i>	1
<i>Escherichia coli</i>	>400
<i>Histophilus somni</i>	25
<i>Klebsiella pneumoniae</i>	32
<i>Ornithobacterium rhinotracheale</i>	≤64
<i>Pasteurella multocida</i>	32
<i>Proteus</i> spp.	>128
<i>Pseudomonas aeruginosa</i>	>256
<i>Salmonella</i> spp.	≤64
<i>Taylorella equigenitalis</i>	4
Mycoplasmas	
<i>M. bovis</i>	4
<i>M. bovis genitalium</i>	4
<i>M. hyopneumoniae</i>	1
<i>M. hyorhinis</i>	1
<i>M. hyosynoviae</i>	4

(daily for 3–5 days) to treat bovine respiratory disease caused by *Mannheimia hemolytica* and *Pasteurella multocida*. It has been used successfully to treat *Salmonella dublin* infection in calves at a dosage of 22 mg/kg SC on the first day and 0.5 g PO twice daily for an additional 4 days (Cook, 1973). Combined the lincomycin, the drug was effective in treating *Ureaplasma* infection in rams (Marcus et al., 1994).

In pigs, spectinomycin is available as an oral preparation for the treatment of colibacillosis. It is also administered IM for the treatment of respiratory disease, including *A. pleuropneumoniae*. Resistance has limited use for this latter purpose. While not approved for this use, IM injection of 10 mg/kg BID for 3 days has been used successfully to treat pigs severely affected with proliferative intestinal adenomatosis. The MIC of spectinomycin against *Lawsonia intracellularis* (32 μg/ml) is the lowest among the aminoglycosides but suggests that the organism is barely susceptible, at least in vitro (McOrist et al., 1995). Spectinomycin is available combined with lincomycin for the oral treatment of swine dysentery, and the combination is also effective

tive for porcine proliferative enteropathy (McOrist et al., 2000).

In dogs, spectinomycin has been administered by IM injection for a variety of infections from Gram-negative bacteria, but no reports of efficacy are available. It is available combined with lincomycin for the treatment of streptococcal, staphylococcal, *Mycoplasma* and *Pasteurella* infections, in dogs and cats with dosage based on 20 mg/kg IM of the spectinomycin component administered once or twice daily. The combination is effective for the treatment of tonsillitis, conjunctivitis, laryngitis, and pneumonia in dogs.

In poultry, spectinomycin is used parenterally in young poult as a single injection to control salmonellosis, pasteurellosis (fowl cholera), *E. coli*, and *Mycoplasma synoviae*. Spectinomycin can be administered in the water to control mortality associated with chronic respiratory disease and infectious synovitis in chickens. The activity of spectinomycin against mycoplasma is a particularly useful attribute but it is surprising that the drug administered orally would have any effect on systemic infections, since it is at best poorly absorbed from the intestine.

Bibliography

- Cook B. 1973. Successful treatment of an outbreak of *Salmonella dublin* infection in calves using spectinomycin. *Vet Rec* 93:80.
- Francoz D, et al. 2005. Determination of *Mycoplasma bovis* susceptibilities against six antimicrobial agents using the E test method. *Vet Microbiol* 105:57.
- Genetsky R, et al. 1994. Intravenous spectinomycin-associated deaths in feedlot cattle. *J Vet Diagn Invest* 6:266.
- Goren E, et al. 1988. Therapeutic efficacy of medicating drinking water with spectinomycin and lincomycin-spectinomycin in experimental *Escherichia coli* infection in poultry. *Vet Quart* 10:191.
- Marcus S, et al. 1994. Lincomycin and spectinomycin in the treatment of breeding rams contaminated with ureaplasmas. *Res Vet Sci* 57:393.
- McOrist S, et al. 1995. Antimicrobial susceptibility of ileal symbiont intracellularis isolated from pigs with proliferative enteropathy. *J Clin Microbiol* 33:1314.
- McOrist S, et al. 2000. Therapeutic efficacy of water-soluble lincomycin-spectinomycin powder against porcine proliferative enteropathy in a European field study. *Vet Rec* 146(3):61.
- Vaillancourt J-P, et al. 1988. Changes in the susceptibility of *Actinobacillus pleuropneumoniae* to antimicrobial agents in Quebec (1981–1986). *J Am Vet Med Assoc* 193:470.

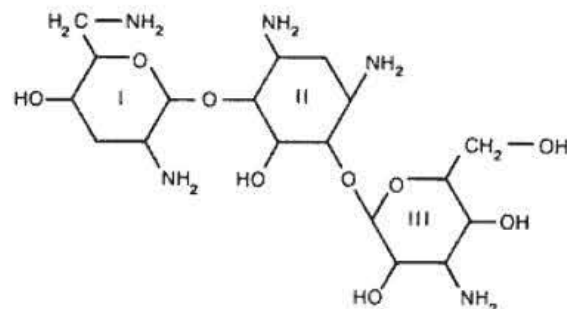


Figure 13.4. Structural formula of tobramycin.

Welsh RD, et al. 2004. Isolation and antimicrobial susceptibilities of bacterial pathogens from bovine pneumonia: 1994–2002. *J Vet Diagn Invest* 16:426.

Tobramycin

Tobramycin is a naturally occurring deoxykanamycin (Figure 13.4) with antimicrobial and pharmacokinetic properties similar to gentamicin. Tobramycin is structurally related to kanamycin and has four times the activity of gentamicin against *Pseudomonas* spp. Tobramycin is generally not effective against gentamicin-resistant strains of Enterobacteriaceae. For treatment of serious *P. aeruginosa* infections, tobramycin should be combined with an antipseudomonal penicillin. One other advantage of tobramycin over gentamicin is its reduced nephrotoxicity, although ototoxic properties are similar. In a study of tobramycin pharmacokinetics in cats, Jernigan et al. (1988) found persistent elevations of blood urea nitrogen and serum creatinine, suggesting possible renal damage 3 weeks after a single dose (5 mg/kg) of tobramycin. The authors suggested that this high dose may have occupied and saturated binding sites in the kidneys from which the drug was only slowly released. Blood urea nitrogen rose in fewer cats after a lower dose (3 mg/kg). Besides evidence of renal toxicity, there was also evidence of dose-dependent differences in pharmacokinetics, suggesting that further studies of toxicity and pharmacokinetics are required in multiple-dosing studies before tobramycin can be recommended in cats. Currently, due to the expense of systemic therapy, tobramycin use in veterinary medicine is mainly limited to the ophthalmic formulation in the treatment of bacterial keratitis due to *P.*

aeruginosa. Emerging resistance to tobramycin has been documented in equine corneal infections (Sauer et al., 2003). Tobramycin has also been used in antibiotic-impregnated polymethyl methacrylate beads for the treatment of septic arthritis or osteomyelitis in horses (Holcombe et al., 1997).

Bibliography

- Holcombe SJ, et al. 1997. Use of antibiotic-impregnated polymethyl methacrylate in horses with open or infected fractures or joints: 19 cases (1987–1995). *J Am Vet Med Assoc* 211:889.
- Jernigan AD, et al. 1988. Pharmacokinetics of tobramycin in cats. *Am J Vet Res* 49:608.
- Sauer P, et al. 2003. Changes in antibiotic resistance in equine bacterial ulcerative keratitis (1991–2000): 65 horses. *Vet Ophthalmol* 6:309.

Tetracyclines and Glycylcyclines

Steeve Giguère

Chlortetracycline was discovered in 1944 and marketed for clinical use in 1948. This was followed shortly thereafter with the discovery and marketing of oxytetracycline. These were the first members of the tetracycline family approved for clinical use. These molecules, along with the later discovered tetracycline and demecloxycline, are products of *Streptomyces* spp. Semi-synthetic derivatives are also available (methacycline, lymecycline, rolitetracycline, doxycycline, minocycline). Tetracyclines are classic broad-spectrum antibiotics because of their activity against Gram-positive and Gram-negative aerobic and anaerobic bacteria, including such organisms as *Brucella*, *Coxiella*, *Ehrlichia*, *Anaplasma*, some *Mycobacterium*, *Mycoplasma* and *Rickettsia/Neorickettsia*. The extraordinarily widespread nature of acquired resistance, which has developed in the many common pathogens and particularly Gram-negatives, has limited the use of the tetracyclines in many clinical circumstances. They have generally favorable pharmacokinetic properties, particularly the more lipophilic semi-synthetic derivatives. Because of their low cost and the limited choice of antibacterial agents available, they tend to be used as first-line antibiotics in ruminants and swine. With the advent of newer antimicrobial agents and the increasing resistance to tetracyclines, these agents are no longer first-choice antibiotics in companion animals or horses. Despite their limitations and restricted use patterns in some animal species, they are still the drug of choice for the treatment of chlamydophilosis, ehrlichiosis, coxiellosis, and rickettsiosis, and for some mycobacterial and mycoplasmal infections.

Chemistry

The tetracyclines are crystalline amphoteric substances that can exist as acid or base salts. They are

available for use, mainly as the hydrochlorides, in a wide variety of dosage forms, both oral and parenteral. Solutions of the hydrochlorides are acidic and, with the exception of chlortetracycline, fairly stable. All tetracyclines have the same basic structure (Figure 14.1). The tetracyclines are strong chelating agents. This ability to chelate divalent and trivalent metal ions such as calcium may result in tooth discoloration.

Mechanism of Action

The tetracyclines are bacteriostatic antibiotics that inhibit protein synthesis in susceptible microorganisms. After diffusion through the outer cell membrane, an active carrier-mediated process transports the drugs through the inner cytoplasmic membrane. Once inside the cell, the tetracyclines bind reversibly to receptors on the 30S subunit of the bacterial ribosome where they interfere with the binding of the aminoacyl-transfer RNA to the RNA-ribosome complex. This binding prevents the addition of amino acids to the elongating peptide chain, inhibiting protein synthesis.

Antimicrobial Activity

Tetracyclines are classic broad-spectrum antibiotics. They exhibit activity against a broad-spectrum of Gram-positive and Gram-negative bacteria including such microorganisms as mycoplasma, ehrlichia/anaplasma, chlamydia/chlamidophila, and rickettsia/neorickettsia. The spectrum of activity of tetracyclines also encompasses various protozoan parasites such as *Plasmodium falciparum*, *Entamoeba histolytica*, *Giardia lamblia*, *Leishmania major*, *Trichomonas* spp., and *Toxoplasma gondii*.

Tetracycline is typically used as the class drug in determining susceptibility. Minocycline, which is the most lipid soluble, has greater activity than other tet-

	R	R ₁	R ₂
Tetracycline	—H	—CH ₃	—H
Chlortetracycline	—Cl	—CH ₃	—H
Oxytetracycline	—H	—CH ₃	—OH
Demeclocycline	—Cl	—H	—H
Methacycline	—H	=CH ₂ *	—OH
Doxycycline	—H	—CH ₃	—OH
Minocycline	—N(CH ₃) ₂	—H	—H

*No hydroxyl at C6.

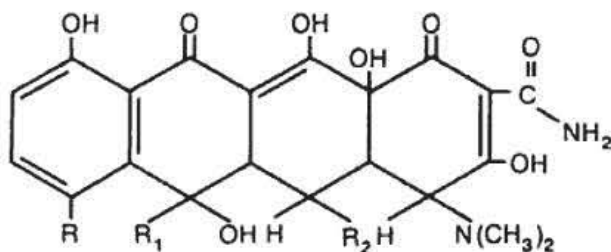


Figure 14.1. Structural formulas of tetracyclines.

racyclines against *Nocardia*, some mycobacteria, and anaerobes. Generally, doxycycline and minocycline are more active against *Staphylococcus aureus* and various streptococci than is tetracycline.

Good or moderate activity (MIC ≤ 4 $\mu\text{g/ml}$)

The tetracyclines, as a group, exhibit good to moderate activity against the following Gram-positive aerobes: *Bacillus* spp., *Corynebacterium* spp., *Erysipelothrix rhusiopathiae*, *Listeria monocytogenes*, some streptococci and against the following Gram-negative bacteria: *Actinobacillus* spp., *Bordetella* spp., *Brucella* spp., *Francisella tularensis*, *Haemophilus* spp., *Lawsonia intracellularis*, *Pasteurella* spp., including *P. multocida*, *Mannheimia* spp., *Yersinia* spp., *Campylobacter fetus*, *Borrelia* spp., *Leptospira* spp. (Table 14.1), *Mycoplasma* spp., *Chlamydia/Chlamidophila* spp., *Rickettsia/Neorickettsia*, *Coxiella burnetii*, *Ehrlichia* spp., *Anaplasma* and some anaerobes including *Actinomyces* spp., and *Fusobacterium* spp.

Variable susceptibility

Because of acquired resistance, many isolates of staphylococci; enterococci; streptococci; Enterobacteriaceae including *Enterobacter* spp., *E. coli*; *Klebsiella* spp.; *Proteus* spp.; and *Salmonella* spp. and anaerobes such as *Bacteroides* spp. and *Clostridium* spp. show

variable susceptibility. Some isolates of *Mannheimia haemolytica* may also be resistant to tetracyclines.

Resistant (MIC ≥ 16 $\mu\text{g/ml}$)

Most *Mycobacterium* spp., *Proteus mirabilis*, *P. aeruginosa*, *Serratia* spp.; some *Mycoplasma* spp. are resistant.

Resistance

Acquired resistance to tetracyclines is widespread among bacteria and *Mycoplasma* and has considerably reduced the usefulness of these agents (Table 14.1). Resistance is extremely rare amongst obligate intracellular pathogens such as *Chlamydia*, *Chlamydophila*, *Ehrlichia* and *Anaplasma*. However, horizontal transmission of tetracycline resistance was recently demonstrated in *Chlamydia suis* (Dugan et al., 2004). Most tetracycline resistant bacteria carry one or more of the 36 different acquired tetracycline resistance genes (Roberts, 2003). These genes are often on mobile elements such as plasmids, transposons, conjugative transposons, and/or integrons.

Resistance to tetracyclines can be mediated by one of three different mechanisms: (1) an energy-dependant efflux of tetracyclines carried out by transmembrane spanning proteins, which results in reduction of the concentration of tetracyclines in the cytosol; (2) ribosomal protection, in which the tetracyclines no longer bind productively to the bacterial ribosome; or (3) chemical modification, requiring oxygen, NADPH, and catalysis by enzymes. The first two mechanisms are by far the most common.

Pharmacokinetic Properties

In dogs and cats, most tetracyclines are adequately absorbed from the gastrointestinal tract, but systemic availability can vary widely among different oral preparations. With the exceptions of minocycline and doxycycline, the absorption of tetracyclines is decreased by the presence of food (particularly milk and its products) in the stomach. Divalent and trivalent cations (calcium, magnesium, iron, aluminum) decrease absorption by chelating tetracyclines.

The drugs vary in lipid solubility, and this property largely determines their distribution and rate of excretion. They enter most tissues and body fluids with the exception of cerebrospinal fluid (CSF). The rate at which they penetrate the blood-brain and blood-CSF barriers is related to their degree of lipid solubility and, to a much lesser extent, plasma protein-binding.

Table 14.1. In vitro activity (MIC₉₀, µg/ml) of tetracycline against bacteria, including *Mycoplasma*.

Organism	MIC ₉₀	Organism	MIC ₉₀
Gram-positive aerobes			
<i>Arcanobacterium pyogenes</i>	16	<i>Staphylococcus aureus</i>	>64
<i>Bacillus anthracis</i>	4	<i>Streptococcus agalactiae</i>	0.25
<i>Corynebacterium pseudotuberculosis</i>	≤ 0.25	<i>S. dysgalactiae</i>	> 32
<i>C. renale</i>	4	<i>S. suis</i>	64
<i>Erysipelothrix rhusiopathiae</i>	0.25	<i>S. uberis</i>	0.5
<i>Listeria monocytogenes</i>	1	<i>S. equi</i> (ssp. zooepidemicus and equi)	> 16
<i>Rhodococcus equi</i>	8		
Gram-negative aerobes			
<i>Actinobacillus</i> spp.	≤ 0.25	<i>Klebsiella pneumoniae</i>	≥ 16
<i>A. pleuropneumoniae</i>	≥ 16	<i>Moraxella bovis</i>	1
<i>Bordetella avium</i>	≥ 16	<i>Manheimia haemolytica</i>	≥ 16
<i>B. bronchiseptica</i> (pig)	≥ 16	<i>Pasteurella</i> spp. (horse)	≤ 2
<i>Brucella canis</i>	0.25	<i>P. multocida</i> (pig)	1
<i>Campylobacter fetus</i>	2	<i>Proteus</i> spp.	≥ 16
<i>C. jejuni</i>	≥ 64	<i>Pseudomonas</i> spp.	≥ 16
<i>Escherichia coli</i>	≥ 64	<i>Salmonella</i> spp.	≥ 16
<i>Haemophilus parasuis</i>	0.5	<i>Tylorella equigenitalis</i>	0.5
<i>Histophilus somni</i>	2		
Anaerobes			
<i>Actinomyces</i> spp.	1	<i>Clostridium</i> spp.	8
<i>Bacteroides fragilis</i>	2	<i>C. perfringens</i>	32
<i>Bacteroides</i> spp.	25	<i>C. difficile</i>	16
<i>Fusobacterium necrophorum</i>	4	<i>Dichelobacter nodosus</i>	0.12
Mycoplasma			
<i>Mycoplasma bovirhinis</i>	0.5*	<i>M. hyorhinis</i>	2
<i>M. bovis</i>	4*	<i>M. hyosynoviae</i>	32
<i>M. canis</i>	16	<i>M. ovipneumoniae</i>	0.5
<i>M. hyopneumoniae</i>	0.03	<i>Ureaplasma</i> spp.	0.06
<i>M. agalactiae</i>	0.5		
Spirochetes			
<i>Borrelia burgdorferi</i>	1		
<i>Leptospira</i> spp.	4		

* Some reports show resistance

Minocycline and doxycycline are more lipid soluble than other members of the group and are also more highly bound to plasma proteins. The limited evidence available suggests that minocycline has greater capacity than other tetracyclines to penetrate cellular barriers, as it attains higher concentrations in poorly accessible fluids such as tears and prostatic fluid. Because tetracyclines have approximately equal antimicrobial activity against most Gram-negative bacteria, the higher concentrations attained in tissues by minocycline give this congener greater clinical efficacy. For Gram-positive bacteria, minocycline and doxycycline are more active than the other tetracyclines. As a result of chelation with calcium, tetracyclines become bound

at active sites of ossification and in developing teeth. The drugs cross the placenta to reach the fetus and are secreted in milk, where they reach concentrations approximating those of serum.

The tetracyclines, except minocycline and doxycycline, are excreted unchanged in urine and, to a lesser extent, bile. As glomerular filtration is their mechanism of excretion, impaired renal function can increase their elimination half-life. Tetracyclines undergo enterohepatic circulation, with much of the compound excreted in bile being reabsorbed from the intestine. This process contributes to the half-life of 6–10 hours, which is long for drugs that are eliminated mainly by renal excretion.

The cumulative recovery of minocycline from urine and feces is much lower than for other tetracyclines, which suggests that this compound is eliminated partly by metabolism. The elimination mechanism for doxycycline differs from that of other tetracyclines in that this agent enters the intestine both by excretion in the bile and directly by diffusion and is excreted in feces. Because doxycycline elimination does not involve renal excretion, it can be used for the treatment of systemic infection in dogs and cats with renal impairment.

The bioavailability of orally administered oxytetracycline is 5% compared to 37% for chlortetracycline in non-fasting calves. Bioavailability is further reduced by administration in milk or milk replacer. Serum concentrations of tetracyclines are much higher following oral administration to fasted calves. The long-acting formulations of oxytetracycline available for IM administration to food animals have their long-acting effect because of both the high dosage used and the prolonged drug persistence at the site of IM injection as a result of tissue irritation (Nouws et al., 1990).

In swine, the bioavailability of most orally administered tetracyclines is poor. Because of this, and the fact that tetracyclines are time-dependent drugs (see Chapter 5), the achievable serum concentrations are only adequate for treating the most susceptible organisms following administration in food or water. However, because of increased rates of absorption, therapeutic concentrations of doxycycline or minocycline may be reached by administration of high concentrations of the drug in feed.

The pharmacokinetics of IV and IM oxytetracycline and oral doxycycline have been studied in horses. Intravenous administration of oxytetracycline is preferred over IM administration because it results in higher and more persistent serum concentrations. The usefulness of oral doxycycline in horses is limited due to the low serum concentrations achieved, presumably as a result of poor oral bioavailability of the drug. For example, the average peak serum concentration in horses following multiple administrations at a dose of 10 mg/kg at 12 h intervals is only 0.46 µg/mL. This is in contrast to peak serum concentrations of 3.5 µg/mL in dogs receiving a dose of 5 mg/kg. Concentrations in synovial and peritoneal fluids of horses are similar to peak serum concentrations, while the drug cannot be detected in CSF. Endometrial tissue concentrations are slightly higher than serum concentrations, while concentrations in urine range between 75 and 145 µg/mL.

Drug Interactions

Absorption of tetracyclines is impaired by antacids containing aluminum, calcium, or magnesium, by iron-containing preparations, and by bismuth subsalicylate. Synergism between tetracyclines and tylosin against *Pasteurella* has been described and may occur with other macrolides and other bacteria. Combination with polymyxins may also give synergistic effects by enhancing bacterial uptake of the drugs. Doxycycline is synergistic with rifampin or streptomycin in the treatment of brucellosis. Doxycycline was synergistic with pyrimethamine in the effective treatment of toxoplasmosis in experimentally infected mice.

Toxicity and Adverse Effects

From a pharmacologic viewpoint, the tetracyclines are relatively safe drugs. Adverse effects may be attributed to their severely irritant nature (vomiting after oral administration, tissue damage at injection site), their disturbance of intestinal flora, their ability to bind calcium (cardiovascular effects, deposition in teeth and bone), and their toxic effects on liver and kidney cells. Fatal anaphylaxis has occasionally been recorded. Unless administered slowly, IV injection of a tetracycline is likely to cause an animal to collapse. It is postulated that this is due to the cardiovascular effect of the high initial concentration in the systemic circulation. Severe renal tubular damage has been attributed to the administration of outdated or improperly stored preparations and is due to byproducts of degradation.

Although not well documented in veterinary medicine, tetracyclines induce dose-related functional changes in renal tubules in several species (Riond and Riviere, 1989). Tetracycline-induced renal toxicosis may be exacerbated by dehydration, hemoglobinuria, myoglobinuria, toxemia, or the presence of other nephrotoxic drugs (Riond and Riviere, 1989).

Severe liver damage can follow overdosage of tetracyclines in animals with pre-existing renal failure and may also be associated with late pregnancy. In cattle, high doses (33 mg/kg IV) have led to fatty infiltration of the liver and severe proximal renal tubule necrosis (Griffin et al., 1979). Tetracyclines should be administered to cattle only in recommended doses to avoid problems of nephrotoxicosis (Lairmore et al., 1984). Transient hemoglobinuria with trembling and subnormal temperatures lasting four hours has been reported with long-acting formulations (Anderson,

Table 14.2. Usual dosages of tetracyclines in selected domestic animal species.

Species	Drug	Route	Dose (mg/kg)	Interval (h)	Comments
Dogs and cats	Chlortetracycline, oxytetracycline	PO	20	8	
	Oxytetracycline	IV	10	12	Slow IV
	Doxycycline, minocycline	PO, IV	5-10	12	Slow IV
Horses	Oxytetracycline	IV	5	12	Slow IV
	Doxycycline	PO	10	12	
Ruminants	Oxytetracycline, tetracycline	IM, IV	10	12-24	Slow IV
	Long acting	IM	20	48	
Swine	Oxytetracycline, tetracycline	IM	10-20	12-24	
	Long acting	IM	20	48	
	Doxycycline	PO	10	12	

1983). Malabsorption because of moderate diarrhea may occur in calves after oral administration of therapeutic doses. Rapid IV administration in cattle has been followed by collapse, probably the result of calcium binding and consequent cardiovascular depression (Gyrd-Hansen et al., 1981), although the propylene glycol vehicle may be responsible. Intravenous injections of all forms of tetracyclines should be given slowly to cattle over a period of not less than 5 minutes (Gyrd-Hansen et al., 1981). In horses, the most feared side effect of tetracyclines is enterocolitis due to alteration of intestinal microflora and superinfection with resistant *Salmonella* or unidentified pathogens which may include *Clostridium difficile* or *C. perfringens*. This occurs in only a small percentage of treated horses. Intravenous administration of doxycycline to horses results in cardiovascular collapse and death, even when slow IV infusions are used. Nephrotoxicosis has been reported especially in foals receiving high doses for the treatment of contracted tendons (see below).

In dogs, fatal nephrotoxicosis has been reported after the IV administration of tetracyclines at higher than recommended dosages. Administration to growing puppies or pregnant bitches results in yellow discoloration of primary and, to a lesser extent, permanent teeth.

Oxytetracycline irritates tissues. Marked differences have been found in the different formulations of oxytetracyclines in this respect (Nouws et al., 1990). The more irritating the product, the lower the bioavailability and the greater the associated drug persistence at the injection site (Nouws, 1984). The long-acting 20% formulations are particularly irritating. The prolonged action is related to delayed release and hence is associated with increased irritation.

Tetracyclines have anti-anabolic effects that may produce azotemia. Such effects can be exacerbated by corticosteroids. The drugs may also cause metabolic acidosis and electrolyte imbalance.

Administration and Dosage

Recommended dosages are shown in Table 14.2. Tetracyclines are available both in capsular and tablet forms and are usually administered PO to dogs and cats. Milk, antacids, and ferrous sulfate interfere with absorption.

Because of poor water solubility, oxytetracycline dihydrate must be given in much higher doses than the hydrochloride to produce equivalent tissue concentrations. Intramuscular injection of tetracyclines cannot be recommended for horses or companion animals because of local tissue damage and pain, and erratic absorption. The recommended dose in cattle is 10 mg/kg given IM or preferably IV, because of variability in absorption. The long-acting oxytetracycline parenteral preparation, which is oxytetracycline base in 2-pyrrolidone, is approved for IM use in cattle and swine only. A single IM dose of 20 mg/kg provides serum concentrations of oxytetracycline above 0.5 µg/ml for 48 hours but appears to offer no advantage over the same dose of the conventional drug IM (Nouws, 1986). Subcutaneous injection in cattle maintains similar serum concentrations to those following IM administration and appears to be better tolerated. To prevent adverse effects, it is important to differentiate between the conventional and the long-acting formulation in dosage decisions.

Clinical Applications

The primary indications for tetracyclines are in the treatment of borreliosis, brucellosis, chlamydiosis,

ehrlichiosis, leptospirosis, listeriosis, rickettsiosis, and tularemia. The older tetracyclines have been used for many years in managing infectious diseases in food animals because of their low cost, broad antimicrobial activity, ease of administration, and general effectiveness. However, their widespread use has undoubtedly contributed to resistance in important pathogenic bacteria, which now limits their value. The tetracyclines' capacity to attain effective concentrations in most tissues, except those separated by specialized cellular barriers, together with their broad-spectrum of activity, makes them particularly useful in the treatment of mixed bacterial infections. The activity of the agents against *Rickettsia*, *Chlamydia*, *Ehrlichia*, and some *Mycoplasma* makes them the drugs of choice in treatment of infections caused by these microorganisms. Although recommended for the treatment of plague, results in the treatment of experimental infections in animals have sometimes been disappointing. The lipophilic character of the newer tetracyclines (minocycline, doxycycline) allows them to attain concentrations in sites such as the prostate, which are poorly accessible to older members of the group. One disadvantage of tetracyclines over a number of other antimicrobial drugs is their bacteriostatic action, so that treatment may need to be for longer than with bactericidal drugs.

Tetracyclines are commonly used in the treatment of brucellosis, usually in combination with rifampin or streptomycin. Doxycycline and minocycline are more effective than older tetracyclines because of better penetration into cells. Treatment with doxycycline should last 6 weeks and with streptomycin 7–14 days. Tetracyclines (particularly minocycline and doxycycline) are also used in the treatment of infections caused by other intracellular bacteria, including *Ehrlichia* and *Coxiella*.

Cattle, Sheep, and Goats

Many of the microorganisms that cause bovine pneumonia are susceptible to tetracyclines at concentrations that can be achieved in lung tissue. The drugs are generally useful in the treatment of bovine pneumonias and also in their prophylaxis, especially in feedlots. Nevertheless, increasing resistance in *Mannheimia haemolytica* and variable susceptibility of *Mycoplasma bovis* limits their effectiveness. The long-acting parenteral formulation, which must be administered by IM injection (or in some formulations, SC), 20 mg/kg

at 48-hour intervals on two to four occasions, may be adequate in treating lower respiratory disease in cattle, sheep, and goats.

If tetracyclines are administered orally to feedlot cattle in the prophylaxis of pneumonia, they should be given in feed and not water. Administration in water may increase mortality (Martin et al., 1982), possibly because of the difficulty of ensuring that even amounts are ingested. While prophylactic administration of drug in the ration appears often to reduce pneumonia and to improve growth and feed conversion efficiency, the cost-to-benefit ratio may not justify this approach. In addition, such a practice tends to promote resistance among *Mannheimia* organisms. In prophylaxis of feedlot pneumonia, parenteral administration gives better effects than oral administration because of higher bioavailability. An approach shown to be useful is to inject tetracyclines when animals enter feedlots or to inject a single dose of long-acting tetracycline to all animals as soon as some in the lots appear to be developing pneumonia.

Clostridial infections and listeriosis can be treated by tetracyclines. A recommended dosage in neural listeriosis is 10 mg/kg/day IV, but clinical trials are needed to determine whether the same dose given twice daily or the use of ampicillin or penicillin G might not be more effective. In listeriosis, IV administration of the conventional preparation (parenteral aqueous solution) is preferred. In human medicine, minocycline is a recognized alternative to ampicillin.

Oxytetracycline is the drug of choice in acute *Anaplasma marginale* infections. However, short term therapy with oxytetracyclines fails to clear the *A. marginale* infections in carrier cattle (Coetzee et al, 2005). Taylor et al. (1986) showed the effectiveness of long-acting tetracyclines in preventing *Babesia bovis* and *B. bigemina* (redwater) in cattle. Tetracyclines are used in the treatment of, and prevention against, heartwater disease caused by *Ehrlichia ruminantium* (Simpson et al., 1987; Mebus and Logan, 1988). The drugs are also used in the prophylaxis of East Coast fever caused by *Theileria parva* (Chumo et al., 1989) and tick-borne fever caused by *Anaplasma phagocytophilum* (Cranwell, 1990).

For infectious keratoconjunctivitis in cattle, 2 doses of the long-acting preparation given 3 days apart can be recommended (George et al., 1988). Long-acting tetracyclines produced moderate cure rates in cattle with dermatophilosis. Long-acting tetracyclines (at

3–4 day intervals for five treatments) combined with streptomycin (IM daily for 7 days) successfully treated 14 of 18 cows with *B. abortus* infection (Nicoletti et al., 1985). Administration once daily as a topical spray (25 mg/ml) was effective in controlling bovine papillomatous digital dermatitis, the efficacy increasing with an increasing number of days of applications (Shearer and Elliott, 1998).

Tetracyclines achieve milk concentrations approximating those of blood, but because of poor bioavailability after IM injection, they are best given IV. They are second-choice parenteral antibiotics for serious infections of the udder caused by Gram-positive bacteria and possibly by coliforms, although susceptibility among the latter organisms is uncommon. Repeated intramammary administration of tetracycline in combination with tylosin cured experimentally induced *Mycoplasma californicum* mastitis in cows (Ball and Campbell, 1989).

In enzootic abortion in sheep caused by *Chlamydia abortus*, experimental and field evidence suggests that two treatments of 20 mg/kg of the long-acting preparation at 2-week intervals, starting 6 to 8 weeks before lambing, will reduce the prevalence of abortions. The drug may be most useful at the start of outbreaks (Greig and Linklater, 1985). Tetracycline is the drug of choice in the prevention and treatment of Q fever (*Coxiella burnetii*). Lambs can be protected from the rickettsial agent of tick-borne fever and associated infections by a single injection of long-acting tetracycline formulation (Brodie et al., 1986). Duration of the effect is between 2 and 3 weeks (Brodie et al., 1988). A single injection of long-acting tetracycline with topical tetracycline is an effective treatment of ovine keratoconjunctivitis caused by *Mycoplasma conjunctivae* (Hosie, 1988; Hosie and Greig, 1995). Long-acting oxytetracycline was highly successful in preventing *M. haemolytica* pneumonia in sheep (Appleyard and Gilmour, 1990), and has been used successfully in the treatment of ovine footrot (Grogono-Thomas et al., 1994), and dermatophilosis (Jordan and Venning, 1995).

Long-acting tetracyclines combined with streptomycin were shown to successfully treat about 80% or more of rams with *Brucella ovis* infection (Marin et al., 1989; Dargatz et al., 1990). Daily intraperitoneal injections of 1000 mg oxytetracycline hydrochloride eliminated *Brucella melitensis* infection from sheep (Radwan et al., 1989).

Swine

Tetracyclines are commonly used in swine to prevent and treat atrophic rhinitis and lower respiratory disease (*Actinobacillus pleuropneumoniae*, *Mycoplasma hyopneumoniae*, *P. multocida*) and to prevent proliferative adenomatosis. Field outbreaks of *Pasteurella* pneumonia have been controlled by feed medication (200–400 g/ton). Feed medication with chlortetracycline, 100 g/ton, has been used to control adenomatosis and at a much higher level, 800 g/ton, to eradicate *Leptospira* from the kidneys of swine (Stahlheim, 1967; Love and Love, 1977). Tetracyclines may be effective against (but because of their bacteriostatic action are not drugs of choice for) erysipelas and *Haemophilus* infections. Enterotoxigenic *Escherichia coli* are usually resistant. Tetracyclines in feed or water have been used successfully to control streptococcal lymphadenitis and *Mycoplasma suis* infection. Orally administered oxytetracycline in pigs had a bioavailability of only 3% in one study, with tetracycline and chlortetracycline being higher (6–18%), particularly in fasted pigs (Nielsen and Gyrd-Hansen, 1996). The use of long-acting formulations in experimentally induced *A. pleuropneumoniae* infections showed that they were more effective than conventional formulations in preventing infections (when administered 48 hours before challenge) but no more effective in treatment. Orally administered doxycycline may have the advantage in pigs of greater bioavailability. An average dose of 11 mg doxycycline/kg bodyweight Q 24 hours in feed for eight days was effective in controlling pneumonia due to *P. multocida* and *M. hyopneumoniae* in pigs (Bousquet et al., 1998).

Horses

The clinical use of oxytetracycline in horses has long been controversial because of early anecdotal reports of severe enterocolitis. While oxytetracycline may, like many other antimicrobial agents, cause enterocolitis, the vast majority of treated horses do not exhibit side effects. Nevertheless, the main factor limiting the use of tetracyclines in horses is their limited spectrum against common equine pathogens.

Oxytetracycline is active in vitro against most non-enteric Gram-negatives of equine origin such as *Pasteurella* spp., and *Actinobacillus* spp. The drug is also active against approximately 70% of *Staphylococcus* spp. However, at clinically achievable concentrations, oxytetracycline is active against only 50–60% of

Enterobacteriaceae and β -hemolytic streptococci. Doxycycline is generally safe when administered orally to horses, but it has poor bioavailability. Oxytetracycline or doxycycline is the treatment of choice for infections caused by *Borrelia burgdorferi*, *Neorickettsia risticii* and *Anaplasma phagocytophilum* in horses. These microorganisms typically have a very low MIC ($< 0.25 \mu\text{g/ml}$). Oxytetracycline is also highly effective in the treatment of *Anaplasma phagocytophilum* and *Neorickettsia risticii* infections in horses (Madigan and Gribble, 1987; Palmer et al, 1992). Administration of oxytetracycline to ponies experimentally infected with *Borrelia burgdorferi* by tick exposure resulted in elimination of persistent infection. In contrast, doxycycline or ceftiofur were inconsistent in eliminating persistent infection in this experimental model (Chang et al 2005). Oxytetracycline or doxycycline has been used successfully in the treatment of infections by *Lawsonia intracellularis* in foals (Sampier et al., 2006).

Dogs and Cats

Tetracyclines are drugs of choice for *Ehrlichia canis*, *Anaplasma phagocytophilum*, and *Rickettsia rickettsii* infections. Doxycycline administered orally to dogs infected with *Rickettsia rickettsii* is effective in preventing the disease or treating acute illness but may not remove the carrier state. In experimental *Brucella canis* infection, the most effective of several treatments, was minocycline (22 mg/kg every 12 hours for 14 days) co-administered with streptomycin (11 mg/kg every 12 hours for 7 days), but effectiveness must be monitored in the laboratory (Flores-Castro and Carmichael, 1978). Field efficacy of tetracycline and streptomycin was 74% in one study (Nicoletti and Chase, 1987). Tetracycline hydrochloride, 10 mg/kg PO every 8 hours, was successful for the treatment of *P. aeruginosa* urinary tract infections in dogs because of the high urine concentrations of the drug attained (Ling et al., 1980). Other indications in dogs include treatment of Lyme borreliosis and leptospirosis. Minocycline delivered in a subgingival local delivery system improved the clinical and microbiologic response in dogs with periodontitis following root scaling and planing (Hayashi et al., 1998). Doxycycline administered orally for three weeks achieved complete remission of about half of canine patients with superficial pyoderma and partial remission in another 40%, but complete remission in only 14% of patients with deep pyoderma and partial remission in another 51% (Bettenay et al., 1998).

Cats suffering from *Chlamydophila felis* infection of the upper respiratory tract and conjunctiva should be treated with tetracyclines for 14 days to eliminate the organism and to remove the latent carrier state. Tetracyclines are drugs of choice for the treatment of *Mycoplasma haemofelis*. Prolonged oral treatment with doxycycline does not eliminate the carrier state in *Bartonella henselae* or *Bartonella clarridgeae* infection (Kordick et al., 1997). Treatment by tetracyclines of a cat with *Yersinia pestis* infection was only temporarily effective (Culver, 1987).

Poultry

Tetracyclines are effective in the treatment of chlamydophilosis if administered for prolonged periods. Tetracycline or chlortetracycline can be administered in 1% medicated feed (45 days), and doxycycline has been administered at 100 mg/kg IM at 5-day intervals on six or seven occasions (Gylsdorff, 1987) or orally twice daily for 20 days. Tetracyclines are also used in the treatment of chronic respiratory disease (*Mycoplasma gallisepticum*) and infectious synovitis (*Mycoplasma synoviae*), as well as of fowl cholera (*P. multocida*). Prolonged administration of oxytetracycline (250 ppm) in feed is required to control *M. gallisepticum* infection in birds. One report noted the surprising efficacy of tetracycline sorbate in the oral treatment of naturally occurring *Aspergillus fumigatus* infection (Roy et al, 1991).

Uses Unrelated to Their Antibacterial Activity

Tetracyclines, particularly doxycycline and minocycline, have a number of effects independent of their antibacterial action including anti-inflammatory properties, immunosuppression, inhibition of lipase and collagenase activity, and wound healing. Experimentally, tetracyclines have protected mice from endotoxin-induced shock by reducing inflammatory cytokine and nitric oxide production. Minocycline is neuroprotective in many experimental models of neurodegenerative diseases, central nervous system injury, and viral encephalitis (Blum et al, 2004). The neuroprotective effect exerted by minocycline is likely due to its anti-apoptotic properties (Richardson-Burns and Tyler, 2005).

Another common use for oxytetracycline is the treatment of contracted tendons in foals. In one study, intravenous administration of oxytetracycline, at a

dose of 44 mg/kg, resulted in a decrease in the angle of the metacarpophalangeal joint for approximately 96 h. These effects may be due to oxytetracycline-induced inhibition of collagen gel contraction by myofibroblasts through a mechanism mediated by matrix metalloproteinase-1 (Arnockzki et al, 2004). Administration of these high doses of oxytetracycline to foals with preexisting renal damage or hypovolemia, or to foals unable to nurse sufficiently, may result in acute renal failure.

Glycylcyclines

The use of tetracyclines for treating bacterial infections has been limited in recent years because of the emergence of resistant isolates. Research to find tetracycline analogues that circumvented these resistance mechanisms has led to the glycylcyclines. Glycylcyclines have potent activity against a wide spectrum of bacteria and may result in rescue of an antibiotic class which appears to be in danger of becoming obsolete because of resistance.

The most developed glycylcycline is tigecycline. Tigecycline resulted from a *t*-butylglycylamino group at the C-9 position of minocycline (Garrison et al, 2005). Tigecycline is only available as an injectable formulation.

Glycylcyclines are active against a variety of bacterial strains expressing tetracycline resistance genes. Tigecyclines binds five times more strongly to ribosomes than minocycline or tetracycline, probably leading to enhanced ribosomal protection against resistance. Tigecycline is active against a broad range of Gram-positive, Gram-negative and anaerobic microorganisms including multidrug-resistant strains of *Staphylococcus* spp. and *Enterococcus* spp. Tigecycline is not active against *Pseudomonas* spp. (Garrison et al, 2005). Several laboratory animal studies describing the efficacy of tigecycline have been published. Phase 2 and phase 3 clinical trials in people are encouraging. In people, nausea and vomiting are the most important side effects. There are currently no published studies evaluating tigecycline in domestic animal species. Its enhanced activity against many pathogens including resistant microorganisms and its parenteral route of administration suggest its largest role may be in treating serious infections in hospitalized patients.

Bibliography

- Anderson WI. 1983. Hemoglobinuria in cattle given long-acting oxytetracycline. *Mod Vet Pract* 64:997.
- Appleyard WT, Gilmour NJL. 1990. Use of long-acting oxytetracycline against pasteurellosis in lambs. *Vet Rec* 126:231.
- Arnockzki SP, et al. 2004. In vitro effects of oxytetracycline on matrix metalloproteinase-1 mRNA expression and on collagen gel contraction by cultured myofibroblasts obtained from the accessory ligament of foals. *Am J Vet Res* 65:491.
- Ball HJ, Campbell JN. 1989. Antibiotic treatment of experimental *Mycoplasma californicum* mastitis. *Vet Rec* 125:377.
- Bettenay SV, et al. 1998. Doxycycline hydrochloride in treatment of canine pyoderma. *Aust Vet Pract* 28:14.
- Blum et al. 2004. Clinical potential of minocycline for neurodegenerative disorders. *Neurobiol Dis* 17:359.
- Brodie TA, et al. 1986. Some aspects of tick-borne diseases of British sheep. *Vet Rec* 118:415.
- Brodie TA, et al. 1988. Prophylactic use of long-acting tetracycline against tick-borne fever (*Cytoecetes phagocytophilia*) in sheep. *Vet Rec* 122:43.
- Bousquet E, et al. 1998. Efficacy of doxycycline in feed for the control of pneumonia caused by *Pasteurella multocida* and *Mycoplasma hyopneumoniae* in fattening pigs. *Vet Rec* 143:269.
- Chang YF. 2005. Antibiotic treatment of experimentally *Borrelia burgdorferi*-infected ponies. *Vet Microbiol* 107:285.
- Chumo RS, et al. 1989. Long-acting oxytetracycline prophylaxis to protect susceptible cattle introduced into an area of Kenya with endemic East Coast fever. *Vet Rec* 124:219.
- Coetzee JF, et al. 2005. Comparison of three oxytetracyclines regimes for the treatment of persistent *Anaplasma marginale* infections in beef cattle. *Vet Parasitol* 127:61.
- Cranwell MP. 1990. Efficacy of long-acting oxytetracycline for the prevention of tick-borne fever in calves. *Vet Rec* 126:334.
- Culver M. 1987. Treatment of bubonic plague in a cat. *J Am Vet Med Assoc* 191:1528.
- Dargatz DA, et al. 1990. Antimicrobial therapy for rams with *Brucella ovis* infection of the urogenital tract. *J Am Vet Med Assoc* 196:605.
- Dugan J, et al. 2004. Tetracycline resistance in *Chlamydia suis* mediated by genomic islands inserted into the chlamydial *inv*-like gene. *Antimicrob Agents Chemother*. 48:3989.
- Flores-Castro R, Carmichael LE. 1978. Canine brucellosis: Current status of methods for diagnosis and treatment. *Gaines Vet Symp* 27:17.
- Garrison et al. 2005. Tigecycline: an investigational glycylcycline antimicrobial with activity against resistant Gram-positive organisms. *Clin Ther* 27:12.
- George L, et al. 1988. Topically applied furazolidone or parenterally administered oxytetracycline for the treatment of infectious bovine keratoconjunctivitis. *J Am Vet Med Assoc* 192:1415.
- Greig A, Linklater KA. 1985. Field studies on the efficacy of a long-acting preparation of oxytetracycline in controlling outbreaks of enzootic abortion of sheep. *Vet Rec* 117:627.

- Griffin DD, et al. 1979. Experimental oxytetracycline toxicity in feedlot heifers. *Bovine Pract* 14:37.
- Grogono-Thomas R, et al. 1994. The use of long-acting oxytetracycline for the treatment of ovine footrot. *Brit Vet J* 150:561.
- Gylsdorff I. 1987. The treatment of chlamydiosis in psittacine birds. *Isr J Vet Med* 43:11.
- Gyrd-Hansen N, et al. 1981. Cardiovascular effects of intravenous administration of tetracycline in cattle. *J Vet Pharmacol Ther* 6:15.
- Hayashi K, et al. 1998. Clinical and microbiological effects of controlled-release local delivery of minocycline on periodontitis in dogs. *Am J Vet Res* 59:464.
- Hosie BD. 1988. Keratoconjunctivitis in a hill sheep flock. *Vet Rec* 122:40.
- Hosie BD, Greig A. 1995. Role of oxytetracycline dihydrate in the treatment of Mycoplasma-associated ovine keratoconjunctivitis in lambs. *Brit Vet J* 151:83.
- Jordan D, Venning CM. 1995. Treatment of ovine dermatophilosis with long-acting oxytetracycline or a lincomycin-spectinomycin combination. *Aust Vet J* 72:234.
- Kordick DL, et al. 1997. Efficacy of enrofloxacin or doxycycline for treatment of *Bartonella henselae* or *Bartonella clarridgeae* infection in cats. *Antimicrob Agents Chemother* 41:2448.
- Lairmore MD, et al. 1984. Oxytetracycline-associated nephrotoxicosis in feedlot cattle. *J Am Vet Med Assoc* 185:793.
- Ling GV, et al. 1980. Urine concentrations of chloramphenicol, tetracycline, and sulfisoxazole after oral administration to healthy adult dogs. *Am J Vet Res* 41:950.
- Love RJ, Love DN. 1977. Control of proliferative haemorrhagic enteropathy in pigs. *Vet Rec* 100:473.
- Madigan JE, Gribble D. 1987. Equine ehrlichiosis in northern California: 49 cases (196 8(1981)). *J Am Vet Med Assoc* 190:445.
- Marin CM, et al. 1989. Efficacy of long-acting oxytetracycline alone or in combination with streptomycin for treatment of *Brucella ovis* infection of rams. *Am J Vet Res* 50:560.
- Martin SW, et al. 1982. Factors associated with mortality and treatment costs in feedlot calves: the Bruce County beef project, years 1978, 1979, 1980. *Can J Comp Med* 46:341.
- Mebus CA, Logan LL. 1988. Heartwater disease of domestic and wild ruminants. *J Am Vet Med Assoc* 192:950.
- Nicoletti P, Chase A. 1987. The use of antibiotics to control canine brucellosis. *Compend Contin Educ Pract Vet* 9:1063.
- Nicoletti P, et al. 1985. Efficacy of long-acting oxytetracycline alone or in combination with streptomycin in the treatment of bovine brucellosis. *J Am Vet Med Assoc* 187:493.
- Nielsen P, Gyrd-Hansen N. 1996. Bioavailability of oxytetracycline, tetracycline, and chlortetracycline after oral administration to fed and fasted pigs. *J Vet Pharm Ther* 19:305.
- Nouws JFM. 1984. Irritation, bioavailability, and residue aspects of ten oxytetracycline formulations administered intramuscularly to pigs. *Vet Quart* 6:80.
- Nouws JFM. 1986. Factors affecting the oxytetracycline disposition kinetics in ruminants—a review. *Ir Vet News* (May):9.
- Nouws JFM, et al. 1990. A comparative study on irritation and residue aspects of five oxytetracycline formulations administered intramuscularly to calves, pigs and sheep. *Vet Quart* 12:129.
- Palmer JE, et al. 1992. Effect of treatment with oxytetracycline during the acute stages of experimentally induced equine ehrlichial colitis in ponies. *Am J Vet Res* 53:2300.
- Radwan AI, et al. 1989. Experimental treatment of *Brucella melitensis* infection in sheep with oxytetracycline alone or combined with streptomycin. *Trop Anim Health Prod* 21:211.
- Richardson-Burns SM, Tyler-KL. Minocycline delays disease onset and mortality in reovirus encephalitis. *Exp Neuro* 192:331.
- Riond J-L, Riviere JE. 1989. Effects of tetracyclines on the kidney in cattle and dogs. *J Am Vet Med Assoc* 195: 995.
- Riond J-L, Riviere JE. 1990. Pharmacokinetics and metabolic inertness of doxycycline in young pigs. *Am J Vet Res* 51:1271.
- Riond J-L, et al. 1990. Comparative pharmacokinetics of doxycycline in cats and dogs. *J Vet Pharm Ther* 13:415.
- Roberts MC. 2003. Tetracycline therapy: an update. *Clin Infect Dis* 36:462.
- Roy S, et al. 1991. Use of tetracycline sorbate for the treatment of *Aspergillus fumigatus* infection in broiler chickens. *Brit Vet J* 147:549.
- Sampier F, et al. 2006. Tetracycline therapy of *Lawsonia intracellularis* enteropathy in foals. *Equine Vet J* 38:89.
- Shaw DH, Rubin SI. 1986. Pharmacologic activity of doxycycline. *J Am Vet Med Assoc* 189:808.
- Shearer JK, Elliott JB. 1998. Papillomatous digital dermatitis: treatment and control strategies: Part I. *Comp Cont Educ Pract Vet* 20:S158.
- Simpson BC, et al. 1987. Protection of cattle against heartwater in Botswana: Comparative efficacy of different methods against natural and blood-derived challenges. *Vet Rec* 120:135.
- Stahlheim OHV. 1967. Chemotherapy of renal leptospirosis in swine. *Am J Vet Res* 28:161.
- Taylor SM, et al. 1986. Inhibition of *Babesia divergens* in cattle by oxytetracycline. *Vet Rec* 118:98.

Chloramphenicol, Thiamphenicol, and Florfenicol

Patricia M. Dowling

Chloramphenicol is a stable, lipid-soluble, neutral compound. It is a derivative of dichloroacetic acid and contains a nitrobenzene moiety. This p-nitro group is associated with idiosyncratic aplastic anemia in humans (Figure 15.1). Thiamphenicol has a similar antibacterial spectrum to chloramphenicol but differs from the parent compound in that the p-nitro group attached to the benzene ring is replaced by a sulfomethyl group. Florfenicol is a structural analogue of thiamphenicol that also lacks the p-nitro group and is more microbiologically active than thiamphenicol. Neither thiamphenicol nor florfenicol is associated with aplastic anemia in humans or any other species, but both are associated with dose-dependent bone marrow suppression.

Chloramphenicol

Mechanism of Action

Chloramphenicol is a potent inhibitor of microbial protein synthesis. It binds irreversibly to a receptor site on the 50S subunit of the bacterial ribosome, inhibiting peptidyl transferase and thereby preventing the amino acid transfer to growing peptide chains and subsequent protein formation. Chloramphenicol also inhibits mitochondrial protein synthesis in mammalian bone marrow cells in a dose-dependent manner.

Antimicrobial Activity

Chloramphenicol is active against a wide range of Gram-positive and Gram-negative bacteria (Table 15.1), against which it is usually bacteriostatic. Anaerobic bacteria are inhibited at usual therapeutic concentrations (0.25–8 µg/ml). Chloramphenicol suppresses rickettsial and chlamydial growth. While my-

coplasma often show susceptibility in vitro, chloramphenicol therapy of mycoplasma pulmonary infections is often ineffective.

Susceptible organisms (MIC ≤ 8 µg/ml) include among Gram-positive aerobic bacteria: *Actinomyces* spp., *Arcanobacterium pyogenes*, *Bacillus anthracis*, *Corynebacterium* spp., *Erysipelothrix rhusiopathiae*, *Listeria monocytogenes*, many *Staphylococcus* spp., and *Streptococcus* spp. Among Gram-negative aerobic bacteria: *Actinobacillus* spp.; *Bordetella bronchiseptica*; *Brucella canis*; Enterobacteriaceae including many *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., and *Salmonella* serotypes; *Haemophilus* spp.; *Histophilus somni*; *Leptospira* spp.; *Moraxella* spp.; *Mannheimia* spp.; and *Pasteurella* spp. All anaerobes (*Bacteroides* spp., *Clostridium* spp., *Prevotella* spp., *Porphyromonas* spp.) are usually susceptible.

Intermediately susceptible organisms (MIC = 16 µg/ml) include *Rhodococcus equi*.

Resistant organisms (MIC ≥ 32 µg/ml) are *Mycobacterium* spp. and *Nocardia* spp. Resistance often emerges in Gram-negative enteric bacteria.

The most frequently encountered mechanism of bacterial resistance to chloramphenicol is enzymatic inactivation by acetylation of the drug by chloramphenicol acetyltransferases (CATs). Acetylation of the hydroxyl groups on chloramphenicol prevents drug binding to the 50S ribosomal subunit. There are also reports of other mechanisms of resistance, such as efflux systems, inactivation by phosphotransferases, and mutations of target sites or permeability barriers (Schwarz et al., 2004). The CAT genes are commonly found on plasmids in Enterobacteriaceae and Pasteu-

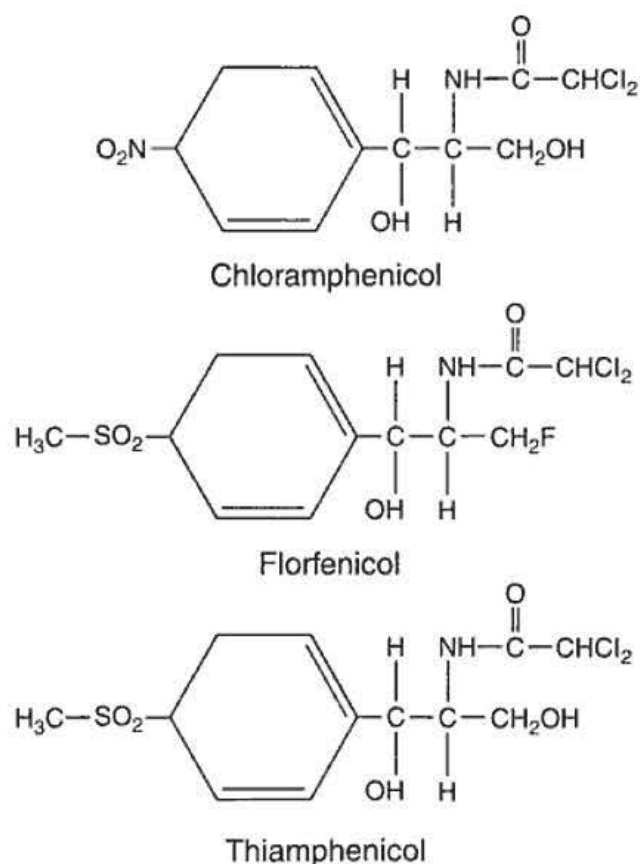


Figure 15.1. Structural formulas of chloramphenicol, florfenicol, and thiamphenicol.

rellaceae, and most of these plasmids carry one or more additional resistance genes. The efflux of chloramphenicol from bacteria can be mediated by either specific transporters or multidrug transporters. Specific transporters tend to have a substrate spectrum limited to a small number of structurally related compounds while the multidrug transporters often have a wide range of unrelated substances as substrates. Specific transporters commonly mediate higher levels of resistance compared to multidrug transporters. Many of the genes coding for the CAT genes or specific exporters are located on mobile genetic elements, such as plasmids, transposons or integrons. When plasmids mediating resistance to chloramphenicol are transferred from one bacterium to another, they are not always able to replicate in the new host. Recombination between the new plasmid and the plasmids already resident in the new host effectively circumvents replication problems. Such recombinations may lead to the formation of novel resistance plasmids which carry

the resistance genes of both parental plasmids and are well adapted to replication in the new host.

Pharmacokinetic Properties

In monogastric animals and preruminant calves, chloramphenicol is typically well-absorbed from the gastrointestinal tract. The oral bioavailability of chloramphenicol in foals is 83%, but only 40% after a single administration in mares, declining to 20% after five doses (Brumbaugh et al., 1983; Gronwall et al., 1986). Chloramphenicol palmitate is poorly absorbed in cats. In ruminants chloramphenicol is inactivated in the rumen. The drug's lipid solubility and its moderately low protein-binding (30–46%) enable it to attain effective concentrations in most tissues and body fluids, including cerebrospinal fluid (CSF) and the central nervous system. Chloramphenicol may achieve CSF concentrations up to 50% of plasma concentrations when the meninges are normal and more if inflammation is present. Topical ophthalmic application results in therapeutic concentrations in the aqueous humour. Chloramphenicol readily diffuses into milk, and pleural and ascitic fluids. It readily crosses the placenta, achieving concentrations 75% of that in maternal plasma. This may be of clinical significance, as the fetal liver is deficient in glucuronyl transferase activity. Penetration of the blood-prostate barrier is relatively poor unless inflammation is present.

Consistent with its lipophilic nature, the apparent volume of distribution of chloramphenicol is large (>1 L/kg) in all species. This can be attributed to widespread distribution, as partitioning of the drug is independent of pH and there is no evidence of selective tissue binding.

The elimination half-life of chloramphenicol varies widely among species. It is short in horses (one hour) (Sisodia et al., 1975) and long in cats (five to six hours) (Watson, 1991). Elimination is primarily by hepatic metabolism by conjugation with glucuronic acid. A fraction of the dose is excreted unchanged by glomerular filtration in the urine of dogs (10%) and cats (20%), while a negligible amount is eliminated by renal excretion in herbivores. The metabolites, which are inactive, are excreted in the urine and to a much lesser extent in the bile. The glucuronide conjugate excreted in bile can be hydrolyzed by intestinal flora to liberate the parent drug.

In newborn animals the elimination half-life of chloramphenicol is considerably longer than in adult

Table 15.1. Activity (MIC₉₀) of chloramphenicol (μg/ml) against selected bacteria and mycoplasma.

Organism	MIC ₉₀	Organism	MIC ₉₀
Gram-positive aerobes			
<i>Acanobacterium pyogenes</i>	1		
<i>Bacillus anthracis</i>	2	<i>Listeria monocytogenes</i>	8
<i>Corynebacterium renale</i>	4	<i>Staphylococcus aureus</i>	8
<i>Enterococcus</i> spp.	>32	<i>Streptococcus dysgalactiae</i>	4
<i>Erysipelothrix rhusiopathiae</i>	2	<i>S. uberis</i>	2
Gram-negative aerobes			
<i>Actinobacillus</i> spp.	4	<i>Klebsiella</i> spp.	>32
<i>Bordetella bronchiseptica</i>	8	<i>Pasteurella</i> spp.	>32
<i>Brucella canis</i>	4	<i>P. multocida</i>	2
<i>Enterobacter</i> spp.	>32	<i>Mannheimia haemolytica</i>	2
<i>Escherichia coli</i>	>32	<i>Proteus</i> spp.	>32
<i>Histophilus somni</i>	1	<i>Pseudomonas aeruginosa</i>	>32
Anaerobes			
<i>Bacteroides</i> spp.	8	<i>Dichelobacter nodosus</i>	0.25
<i>B. fragilis</i>	8	<i>Fusobacterium</i> spp.	1
<i>Clostridium difficile</i>	4	<i>F. necrophorum</i>	2
<i>C. perfringens</i>	4	<i>Brachyspira hyodysenteriae</i>	4
Mycoplasma			
<i>M. bovis</i>	8	<i>M. hyopneumoniae</i>	4
<i>M. bovirhinis</i>	64	<i>M. ovipneumoniae</i>	16
<i>M. canis</i>	8		

animals of the same species. This is due mainly to immature glucuronide conjugation mechanisms. Glucuronide conjugation develops most rapidly in foals, so that the half-life in the one-week-old foal approaches that of the adult horse (Table 15.2).

Drug Interactions

Chloramphenicol should not be used concurrently with a bactericidal drug in treating infections where host defenses are poor. Concurrent chloramphenicol and penicillin G have been shown to be antagonistic in treating bacterial meningitis and endocarditis in humans. Chloramphenicol acts on the same ribosomal site as the macrolides. Chloramphenicol is antagonistic to the fluoroquinolones, as inhibition of protein synthesis by chloramphenicol interferes with the production of autolysins necessary for cell lysis after the fluoroquinolone interrupts bacterial DNA supercoiling.

Because chloramphenicol inhibits microsomal enzyme activity, hepatic metabolism (oxidative reactions and glucuronide conjugation) of drugs given concurrently is slowed, resulting in prolonged pharmacologic effect. Thus chloramphenicol markedly prolongs the

Table 15.2. Pharmacokinetic parameters describing disposition of chloramphenicol (25 mg/kg IV) in foals.

Pharmacokinetic Parameter	Age of Foals (days)			
	1	3	7	14
Half-life* (hrs)	5.29	1.35	0.61	0.51
V _{d(area)} (ml/kg)	1101 ± 284	759 ± 224	491 ± 158	426 ± 65
Cl _B (ml/min per kg)	2.25 ± 0.67	6.23 ± 2.22	8.86 ± 1.90	9.63 ± 1.63

*Denotes harmonic mean. Other data are expressed as mean ± s.d.

From Adamson, 1991.

effect of barbiturates, and fatal effects have been observed in epileptic dogs treated concurrently with phenobarbital (Adams and Dixit, 1970).

Toxicity and Adverse Effects

The main toxic effect of chloramphenicol in humans is bone marrow depression, which can be either an idiosyncratic, non-dose dependent aplastic anemia or a dose-dependent anemia from suppression of protein synthesis. Aplastic anemia is a serious risk associated with chloramphenicol exposure in humans by any

Table 15.3. Usual systemic dosages of chloramphenicol in animals.*

Species	Dosage Form	Route	Dose (mg/kg)	Interval (hrs)	Comments
Dogs, cats	Base, palmitate	Oral	50	12	Limit to 10 days of therapy
	Sodium succinate	IV, IM, SC	25-50	8-12	
Horses	Sodium succinate	IM	30-50	6	
	Base, palmitate	PO	25-50	6-8	

*Owners should be warned of the risks of their exposure to chloramphenicol.

route, as induction of the condition is not dose-related. Aplastic anemia probably represents a genetically determined idiosyncrasy of the individual. The incidence of fatal aplastic anemia has been estimated as one in every 25,000 to 60,000 humans who use the drug. A few cases of aplastic anemia in humans have occurred following contact exposure (ophthalmic use, medicated sprays, handling), so that veterinarians and owners should wear protective gloves and face masks when handling chloramphenicol products (Del Giasco et al., 1981).

A "gray baby" syndrome occurs in newborn infants because their deficiency in glucuronic acid conjugation causes a dose-dependent anemia. In animals, chloramphenicol toxicity is related both to size of dose and duration of treatment, and cats are more likely than dogs to develop toxicity. In cats, clinical signs of toxicity may be seen when the usual maintenance dosage of 25 mg/kg of base or palmitate ester PO twice daily is administered for 21 days (Watson, 1980). Administration of larger doses for this period caused reversible, dose-related disturbances in red cell maturation in the peripheral blood and bone marrow. Administration for less than 10 days using the maintenance dose is not likely to cause toxicity in either dogs or cats, unless the animals have depressed hepatic microsomal enzyme activity or severely impaired renal function. Anaphylaxis, vomiting, and diarrhea have occasionally been reported in dogs and cats treated with therapeutic doses.

Chloramphenicol may suppress antibody production if given prior to an antigenic stimulus and may affect vaccination response.

Administration and Dosage

Recommended drug dosages of chloramphenicol are given in Table 15.3.

Chloramphenicol is a broad-spectrum bacteriosta-

tic drug that can attain effective concentrations at sites of infection that are relatively inaccessible to other antimicrobials. The objective in multiple dosing is to maintain an average steady-state plasma concentration of 5–10 µg/ml.

Chloramphenicol is available for either oral (free base or palmitate ester) or parenteral (sodium succinate) administration. For local treatment of eye or ear infections caused by susceptible organisms, ophthalmic preparations can be applied.

Because the drug is well absorbed from the gastrointestinal tract in small animals, it can be given orally as either the base or the palmitate ester. The ester is hydrolyzed prior to absorption of the free base. The intake of food does not influence bioavailability. Subcutaneous injection of chloramphenicol sodium succinate is an alternative to oral administration. While both routes may provide equivalent concentrations, the oral route is preferable, as injection of the parenteral preparation is painful. The total length of treatment should not exceed 10 days, especially in cats. Do not administer chloramphenicol to patients with evidence of or suspected bone marrow suppression.

The short half-life of chloramphenicol in horses (one hour), together with its generally bacteriostatic action, makes IV administration impractical. An aqueous suspension of the free drug can be administered PO or the sodium succinate given by IM injection. After absorption from injection sites, the inactive succinate ester is rapidly hydrolyzed to the active drug.

Because of the risks of idiosyncratic aplastic anemia in humans, chloramphenicol is banned for use in food animals in most countries. The drug should not be used in the early neonatal period unless plasma concentrations are monitored, and should be used with caution in pregnant animals because of the potential adverse effects on the fetus.

Clinical Applications

The potential for nondose-related fatal aplastic anaemia in humans has led to prohibition of chloramphenicol use in food animals in many parts of the world. Florfenicol is the appropriate analogue to use in food animals. In Britain, concerns over transmissible antimicrobial resistance to human pathogens has also led to restricting use in both farm and companion animals to local treatment of serious eye infections and to systemic conditions for which clinical and laboratory assessment show no safer antibiotic (Joint RCVS/BVA Statement, 1976).

Now that there are a number of fluoroquinolone antimicrobials for companion animals, there are few primary indications for the use of chloramphenicol, but it may be considered for some anaerobic infections, serious ocular infections, prostatitis, otitis media/interna and salmonellosis. Human toxicity from handling chloramphenicol should be discussed with the owner when prescribing the drug for use in dogs and cats.

Thiamphenicol

Thiamphenicol is a derivative of chloramphenicol, in which the *p*-nitro group has been replaced by a sulfo-methyl group. Thiamphenicol is generally one to two times less active than chloramphenicol, although it has equal activity against *Haemophilus*, *B. fragilis*, and streptococci. Cross-resistance with chloramphenicol is complete in bacteria which possess chloramphenicol transacetylases. Absorption and distribution are similar to those of chloramphenicol, and it is also equally well distributed into tissues. Oral bioavailability in pre-ruminant lambs and calves is 60% (Mengozi et al., 2002). Thiamphenicol is not eliminated by hepatic glucuronide conjugation but excreted unchanged in the urine. Unlike chloramphenicol, its elimination is unaffected by liver disease and by the use of other drugs metabolized in the liver. The pharmacokinetic parameters of thiamphenicol follow allometric scaling, in that values for elimination half-life and volume of distribution increase with body size from mice through rats, rabbits, dogs, pigs, sheep and calves (Castells et al., 2001). Therapeutic concentrations are achieved in milk of lactating cows (Abdennebi et al., 1994).

One reason for major interest in thiamphenicol is that, because it lacks the *p*-nitro group, it does not induce irreversible bone marrow aplasia in humans, al-

though it may more commonly cause dose-dependent bone marrow suppression than chloramphenicol.

Thiamphenicol is used extensively in Europe and Japan but is not available in North America. Apart from its bacteriostatic character and lower activity than chloramphenicol, thiamphenicol would appear to have under-exploited potential for use in the treatment of many infections caused by susceptible organisms, because of its advantages of excellent tissue distribution, broad-spectrum of antimicrobial activity, low toxicity, and potential for oral administration. While detailed dosage is not available because of relative lack of pharmacokinetic and clinical studies, suitable dosage in animals would appear to be similar to that of chloramphenicol. Dosages for cattle and pigs are 10–30 mg/kg IM every 24 hours, 30 mg/kg PO every 12 hours for preruminant lambs and every 24 hours for preruminant calves, or 50–200 ppm in feed for pigs and 100–500 ppm in feed for chickens.

Florfenicol

Florfenicol is a fluorinated derivative of thiamphenicol, in which the hydroxyl group at C-3 has been replaced with fluorine. Florfenicol is a potent inhibitor of microbial protein synthesis from the same mechanisms as chloramphenicol. Like thiamphenicol, the drug does not cause idiosyncratic aplastic anaemia but may cause dose-dependent bone marrow suppression if dosed chronically.

Antimicrobial Activity

Florfenicol has a broad range of activity similar to, but slightly more active than, chloramphenicol (Table 15.4). It may have bactericidal activity against *Histophilus somni* and *Pasteurella* spp. The MIC₉₀ for *Actinobacillus pleuropneumoniae*, *Haemophilus* spp.; *Histophilus somni*, *Mannheimia haemolytica*, *Aeromonas hydrophila*, *Pasteurella multocida* and *Streptococcus suis* is ≤ 2 µg/ml. *Fusobacterium necrophorum*, *Provetella melaninogenica* and *Moraxella bovis* are highly susceptible. The MIC₉₀ for Enterobacteriaceae, which are less susceptible, is higher; for example, for *Salmonella dublin* it is 32 µg/ml. Florfenicol is active against a number of important bacterial pathogens of fish including *Aeromonas salmonicida*, *Vibrio salmonicida*, *Vibrio anguillarum* and *Yersinia ruckeri* in salmon and trout and *Edwardsiella ictaluri* in catfish.

Table 15.4. Activity (MIC₉₀; µg/ml) of florfenicol against selected bacteria and mycoplasma.

Organism	MIC ₉₀
Porcine	
<i>Actinobacillus pleuropneumoniae</i>	0.5
<i>Pasteurella multocida</i>	0.5
<i>Bordetella bronchiseptica</i>	8
<i>Streptococcus suis</i>	2
Bovine	
<i>Mannheimia haemolytica</i>	2
<i>Pasteurella multocida</i>	0.5
<i>Arcanobacterium pyogenes</i>	1.56
<i>Salmonella</i> Dublin	32
<i>Mycoplasma bovis</i>	4
<i>Listeria monocytogenes</i>	32.0
Fish	
<i>Edwardsiella ictaluri</i>	0.25
<i>Aeromonas salmonicida</i>	1.6
<i>Vibrio anguillarum</i>	0.5
<i>Photobacterium damsela</i>	0.6
<i>Chryseobacterium</i> spp.	32.0

Because of the substitution of a hydroxyl group with a fluorine molecule, florfenicol is less susceptible to resistance from bacteria expressing chloramphenicol acetyltransferase enzymes. Florfenicol resistance in Gram-negative bacteria has been detected and is related to plasmid transfer of the *flo* gene. This gene codes for a membrane-associated exporter protein that promotes efflux of chloramphenicol and florfenicol (Schwarz et al., 2004). In cases of neonatal calf diarrhea from *E. coli*, if *flo* is present, the MIC range is 16 to ≥ 256 µg/ml (White et al., 2000). The *flo* gene has now been identified in *Pasteurella multocida* isolated from a calf (Kehrenberg and Schwarz, 2005). After a single dose of florfenicol, feedlot cattle show a shift in fecal flora to multi-resistant *E. coli*, likely due to selection for plasmids containing the *flo* gene linked with other resistance genes. The antimicrobial resistance associated with florfenicol treatment declined over four weeks post-treatment, but a higher proportion of fecal *E. coli* were resistant than when the cattle entered the feedlot (Berge et al., 2005).

Pharmacokinetic Properties

The oral bioavailability of florfenicol in horses is 83% and 89% in two to five-week-old calves, but decreases when administered with milk replacers (Varma et al.,

1986). From an IM injection, bioavailability is 81% in horses and 38% in lactating dairy cattle, but 54% with intramammary infusion (McKellar and Varma, 1996; Soback et al., 1995). While values of volume of distribution for florfenicol are slightly lower than for chloramphenicol, florfenicol is well distributed into many tissues including lungs, muscle, bile, kidney and urine. With IV administration, cerebrospinal fluid concentrations were 46% of plasma concentrations, achieving potentially therapeutic concentrations for *H. somni*, but not Gram-negative enteric bacteria (de Craene et al., 1997). With IM administration to beef calves, the serum concentration of florfenicol remains above 1 µg/ml for 22 hours (Lobell et al., 1994). Ten hours after IM administration to dairy cows, milk concentrations peak at 1.6 µg/ml.

The commercially available formulation of florfenicol is long-acting, so that “flip-flop” kinetics occur, where elimination is prolonged due to slow absorption from the injection site. In cattle, 64% of a dose is excreted as parent drug in the urine. Florfenicol metabolites are excreted in urine and feces. Florfenicol amine is the longest-lived metabolite in the liver and is used as the marker residue for withdrawal times.

Drug Interactions

There are no published data on adverse drug interactions with florfenicol. Mechanistically, interactions should be similar to those seen with chloramphenicol.

Toxicity and Adverse Effects

Transient diarrhea or inappetance may occur in cattle, but resolves within a few days of the end of treatment. In swine, perianal inflammation and rectal eversion may occur in treated animals, but should resolve completely within one week after discontinuation of the drug. Reversible bone marrow suppression of protein synthesis in erythroid cells may result from prolonged florfenicol administration. The florfenicol formulation for cattle and swine is only labeled for a maximum of two doses, so bone marrow suppression has not been reported with clinical use in these species. However, a dose-dependent decrease was seen in hematopoietic/lymphopoietic tissue in the anterior kidneys, posterior kidneys, and spleens of channel catfish fed florfenicol for 20 days (Gaikowski et al., 2003). Florfenicol appears safe for use in non-human primates (Cook, et al., 2004).

Administration and Dosage

Florfenicol is approved in numerous countries in beef cattle for the treatment of respiratory disease, pododermatitis, and keratoconjunctivitis caused by highly susceptible bacteria ($MIC \leq 2 \mu\text{g/ml}$) at 20 mg/kg IM twice at a 48-hour interval or 40 mg/kg SC once. Each injection site should not exceed 10 ml. The label dosage does not result in concentrations that would be effective against Gram-negative enteric pathogens. In some countries, florfenicol is approved for the treatment of swine respiratory disease from *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* at 15 mg/kg IM twice at a 48-hour interval. In swine it should be injected into the neck at no more than 5 ml per site. The cattle and swine product is formulated with three different carriers to give the product a long-acting effect from slow absorption.

In Canada, florfenicol is approved for the treatment of furunculosis caused by susceptible strains of *Aeromonas salmonicida* in salmon. In the United States, it is approved for control of catfish mortality due to enteric septicemia associated with *Edwardsiella ictaluri*. In Japan, florfenicol is labeled for the treatment of pseudotuberculosis and streptococcosis in *Perciformes* (yellowtail, amberjack, red sea bream, tilapia etc.) and for the treatment of edwardsiellosis disease in eel. The fish formulation is mixed in unmedicated feed prior to pelleting or used to surface-coat pelleted feed, and fed to deliver 10 mg/kg per day for 10 consecutive days (Gaikowski et al., 2003).

Clinical Applications

Currently, florfenicol is used as an effective treatment for bovine respiratory disease caused by highly susceptible bacteria such as *Mannheimia*, *Pasteurella* and *Histophilus* (Hoar et al., 1998). The same dosage regimen will treat pododermatitis caused by *Fusobacterium necrophorum* and *Provetella melaninogenica* and infectious bovine keratoconjunctivitis caused by *Moraxella bovis*, but penicillin or oxytetracycline are less expensive and narrower in antimicrobial spectrum, and should be used as first-line treatments for these infections. When administered to lactating dairy cows, florfenicol readily crosses into milk. When administered by the intramammary route, bioavailability is higher than when it is administered by IM injection (Soback et al., 1995). Intramammary administration of 750 mg florfenicol in the treatment of bovine mas-

titis caused by a variety of pathogens had no advantage over cloxacillin (Wilson et al., 1996).

Florfenicol at 50 ppm in feed significantly reduced illness due to experimental *Actinobacillus pleuropneumoniae* pneumonia in pigs, including that caused by a strain resistant to thiamphenicol. Two doses of florfenicol at 15 mg/kg q48 hours significantly reduced morbidity caused by the same organism (Jackson et al., 1998; Ueda et al., 1995).

Florfenicol is used in the treatment of susceptible bacterial diseases of fish, including furunculosis in salmon and vibriosis in salmon and cod, pseudotuberculosis in Japanese yellowtail, enteric septicemia in channel catfish and enteric redmouth in trout.

While not approved, florfenicol is used extra-label in a number of species. Pharmacokinetics have been described in sheep, goats, broiler chickens, North American elk, and rabbits (Alcorn et al., 2004; Atef et al., 2001; El-Aty et al., 2004; Lane et al., 2004; Shen et al., 2003).

The use of florfenicol in horses is not recommended. Despite a high oral bioavailability and good distribution, florfenicol administration to horses has been shown to alter fecal consistency following a single dose administered either IV, PO or IM (McKellar and Varma, 1996). In a chronic dosing study using the cattle formulation at 20 mg/kg IM every 48 hours, all horses remained clinically normal but had dramatic alterations in fecal flora (Dowling, 2001).

Bibliography

- Abdennebi EH, et al. 1994. Thiamphenicol pharmacokinetics in beef and dairy cattle. *J Vet Pharmacol Therap* 17:365.
- Adams HR, Dixit BN. 1970. Prolongation of pentobarbital anesthesia by chloramphenicol in dogs and cats. *J Am Vet Med Assoc* 156:902.
- Adamson PJW, et al. 1991. Influence of age on the disposition kinetics of chloramphenicol in equine neonates. *Am J Vet Res* 52:426.
- Alcorn J, et al. 2004. Pharmacokinetics of florfenicol in North American elk (*Cervus elaphus*). *J Vet Pharmacol Ther* 27:289.
- Atef M, et al. 2001. Disposition kinetics of florfenicol in goats by using two analytical methods. *J Vet Med A Physiol Pathol Clin Med* 48:129.
- Berge AC, et al. 2005. Assessing the effect of a single dose florfenicol treatment in feedlot cattle on the antimicrobial resistance patterns in faecal *Escherichia coli*. *Vet Res* 36:723.

- Brumbaugh GW, et al. 1983. Pharmacokinetics of chloramphenicol in the neonatal horse. *J Vet Pharmacol Ther* 6:219.
- Castells G, et al. 2001. Allometric analysis of thiamphenicol disposition among seven mammalian species. *J Vet Pharmacol Ther* 24:193.
- Cook AL, et al. 2004. Use of florfenicol in non-human primates. *J Med Primatol* 33:127.
- Ee Craene BA, et al. 1997. Pharmacokinetics of florfenicol in cerebrospinal fluid and plasma of calves. *Antimicrob Agents Chemother* 41:1997.
- Del Giacco GS, et al. 1981. Fatal bone marrow hypoplasia in a shepherd using chloramphenicol spray. *Lancet* i:945.
- Dowling PM. 2001. Florfenicol in horses: pharmacokinetics and tolerance in horses. 19th Annual ACVIM Forum:198.
- El-Aty AM, et al. 2004. Pharmacokinetics and bioavailability of florfenicol following intravenous, intramuscular and oral administrations in rabbits. *Vet Res Commun* 28:515.
- Gaikowski MP, et al. 2003. Safety of Aquaflo (florfenicol, 50% type A medicated article), administered in feed to channel catfish, *Ictalurus punctatus*. *Toxicol Pathol* 31:689.
- Gronwall R, et al. 1986. Body fluid concentrations and pharmacokinetics of chloramphenicol given to mares intravenously or by repeated gavage. *Am J Vet Res* 47:2591.
- Hoar BR, et al. 1998. A comparison of the clinical field efficacy and safety of florfenicol and tilmicosin for the treatment of undifferentiated bovine respiratory disease of cattle in western Canada. *Can Vet J* 39:161.
- Jackson JA, et al. 1998. Comparative efficacy of florfenicol (FFC) and ceftiofur (CEF) in the treatment of naturally occurring swine respiratory disease. 15th Int Pig Vet Cong, p 187.
- Joint Royal College of Veterinary Surgeons/British Veterinary Association Statement. 1976. Veterinary use of chloramphenicol. *Vet Rec* 99:152.
- Kehrenberg C, Schwarz S. 2005. Plasmid-borne florfenicol resistance in *Pasteurella multocida*. *J Antimicrob Chemother* 55:773.
- Lane VM, et al. 2004. Intravenous and subcutaneous pharmacokinetics of florfenicol in sheep. *J Vet Pharmacol Ther* 27:191.
- Lobell RD, et al. 1994. Pharmacokinetics of florfenicol following intravenous and intramuscular doses to cattle. *J Vet Pharmacol Ther* 17:253.
- McKellar QA, Varma KJ. 1996. Pharmacokinetics and tolerance of florfenicol in Equidae. *Equine Vet J* 28:209.
- Mengozzi G, et al. 2002. A comparative kinetic study of thiamphenicol in pre-ruminant lambs and calves. *Res Vet Sci* 73:291.
- Schwarz S, et al. 2004. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiol Rev* 28:519.
- Shen J, et al. 2003. Bioavailability and pharmacokinetics of florfenicol in broiler chickens. *J Vet Pharmacol Ther* 26:337.
- Shin SJ, et al. 2005. Evaluation of the antimicrobial activity of florfenicol against bacteria isolated from bovine and porcine respiratory disease. *Vet Microbiol* 106:73.
- Sisodia CS, et al. 1975. A pharmacological study of chloramphenicol in horses. *Can J Comp Med* 39:216.
- Soback S, et al. 1995. Florfenicol pharmacokinetics in lactating cows after intravenous, intramuscular and intramammary administration. *J Vet Pharmacol Ther* 18:413.
- Ueda Y, et al. 1995. Efficacy of florfenicol on experimental *Actinobacillus pleuropneumonia* in pigs. *J Vet Med Sci* 57:261.
- Varma KJ, et al. 1986. Pharmacokinetics of florfenicol in veal calves. *J Vet Pharmacol Ther* 9:412.
- Watson AD. 1980. Further observations on chloramphenicol toxicosis in cats. *Am J Vet Res* 41:293.
- Watson ADJ. 1991. Chloramphenicol: (2) Clinical pharmacology in dogs and cats. *Aust Vet J* 68:2.
- White DG, et al. 2000. Characterization of chloramphenicol and florfenicol resistance in *Escherichia coli* associated with bovine diarrhea. *J Clin Microbiol* 38:4593.
- Wilson DJ, et al. 1996. Efficacy of florfenicol for treatment of clinical and subclinical bovine mastitis. *Am J Vet Res* 57:527.

Sulfonamides, Diaminopyrimidines, and Their Combinations

John F. Prescott

The value of the sulfonamides as single antimicrobial agents has been greatly diminished by widespread acquired resistance. However, when combined with antibacterial diaminopyrimidines such as trimethoprim, resistance is diminished and usefulness enhanced.

Sulfonamides

Chemistry

The sulfonamides are derivatives of sulfanilamide, which contains the structural prerequisites for antibacterial activity. The sulfonamides differ in the radical (R) attached to the amido ($-\text{SO}_2\text{NHR}$) group or occasionally in the substituent on the amino ($-\text{NH}_2$) group (Figure 16.1).

The various derivatives differ in physicochemical and pharmacokinetic properties and in degree of antimicrobial activity. As a group, sulfonamides are quite insoluble; they are more soluble at an alkaline pH than at an acid pH. In a mixture of sulfonamides, each component drug exhibits its own solubility. An example is the trisulfapyrimidine preparation, in which the antibacterial activity of the combined sulfonamides is additive, but the agents behave independently with respect to solubility. This mixture was developed to offset the precipitation of sulfonamide crystals in acidic fluid in the distal renal tubules and ureters.

The sodium salts of sulfonamides are readily soluble in water, and parenteral preparations are available for IV injection. These solutions are highly alkaline in reaction, with the notable exception of sodium sulfacetamide, which is nearly neutral and is available as an ophthalmic preparation.

Certain sulfonamide molecules are designed for low solubility (e.g., phthalylsulfathiazole), so they are slowly absorbed and are intended for use in treatment of enteric infections.

Mechanism of Action

Sulfonamides interfere with the biosynthesis of folic acid in bacterial cells by competitively preventing para-aminobenzoic acid (PABA) incorporation into the folic (pteroylglutamic) acid molecule. Specifically, sulfonamides compete with PABA for the enzyme dihydropteroate synthetase. Their selective bacteriostatic action depends on the difference between bacterial and mammalian cells in the source of folic acid. Susceptible microorganisms must synthesize folic acid, whereas mammalian cells use preformed folic acid. The bacteriostatic action can be reversed by an excess of PABA, so that tissue exudates and necrotic tissue should be removed if animals are to be treated with sulfonamides.

Antimicrobial Activity

Sulfonamides are broad-spectrum antimicrobial agents, inhibiting bacteria, toxoplasma and other protozoal agents such as coccidia, but their antibacterial activity is significantly limited by the extensive resistance that has developed after over 50 years of use. Different sulfonamides may show quantitative but not necessarily qualitative differences in activity.

The MIC of sulfonamides is markedly affected by the composition of the medium and the bacterial inoculum concentration. Because of this, in vitro tests may sometimes falsely report a bacterium to be resistant. This will not be the case if proper quality control

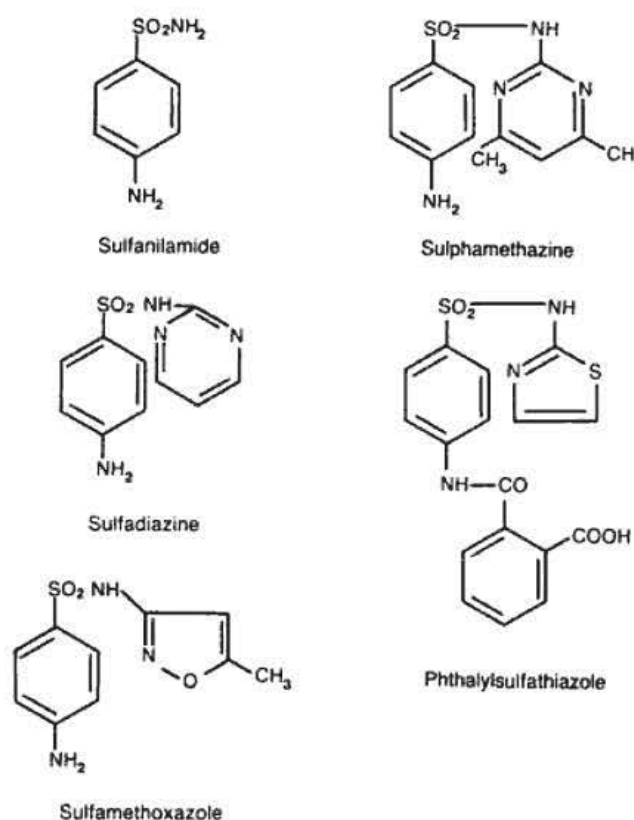


Figure 16.1. Structural formulas of some sulfonamides.

with a thymidine-sensitive strain of *Enterococcus faecalis* is used. In agar diffusion tests, Mueller Hinton agar containing lysed horse blood is the ideal medium because it contains thymidine phosphorylase, which decreases the quantity of thymidine in the medium. The criteria of susceptibility for bacteria in systemic infections are not agreed upon, because of difficulties in determining MIC and because of the variability in serum concentrations with different drugs and different doses. An MIC of 8–32 µg/ml is a reasonable definition of susceptibility for short-acting systemic sulfonamides; an MIC of ≥64–128 µg/ml can be interpreted as evidence of resistance.

Sulfonamide susceptibility testing in veterinary laboratories is usually done with high-potency triple-sulfonamide disks, designed to determine susceptibility to the high concentrations in the urinary tract (≤100 µg/ml); extrapolation of susceptibility to systemic infections is thus not appropriate. The NCCLS criteria describe susceptibility in bacteria for urinary tract infections as those having an MIC of ≤256 µg/ml.

Good susceptibility. *Bacillus* spp., *Brucella* spp., *E. rhusiopathiae*, *L. monocytogenes*, *Nocardia* spp., pyogenic *Streptococcus* spp., *Chlamydia* spp., coccidia, *Pneumocystis carinii*, and *Cryptosporidium* spp.

Moderate susceptibility, but often variable because of acquired resistance (Table 16.1) includes among Gram-positive aerobes: staphylococci, some enterococci. Gram-negative aerobes: Enterobacteriaceae including *Enterobacter* spp., *E. coli*, *Klebsiella* spp., *Proteus* spp., *Actinobacillus* spp., *Haemophilus/Histophilus* spp., *Pasteurella* spp., and *Pseudomonas* spp. Anaerobes such as *Bacteroides* spp. and *Fusobacterium* spp. are often susceptible in vitro if the medium is depleted of thymidine; this, however, is often not the case in vivo. *Clostridium* spp. (other than *C. perfringens*) and anaerobic cocci are often resistant.

Resistant. *Mycobacterium* spp., *Mycoplasma* spp., most obligate intracellular pathogens such as *C. burnetii* and *Rickettsia* spp., *P. aeruginosa*, and spirochetes are resistant.

Resistance

Chromosomal mutation to resistance develops slowly and gradually and results from impairment of drug penetration, production of an insensitive dihydropteroate enzyme, or hyperproduction of PABA. Plasmid- and integron-mediated resistance, often encoded by *sulI* and *sulII* genes sometimes linked to other resistance genes including trimethoprim (*dhfrI*) or streptomycin (*aadA1a*), is far more common and in enteric bacteria is the result of impaired drug penetration or the production of additional, sulfonamide-resistant, dihydropteroate synthetase enzymes (Lanz et al., 2003; Maynard et al., 2003). Resistance to sulfonamides is widespread in bacteria isolated from animals, reflecting extensive use of these drugs over many years. There is complete cross-resistance among the sulfonamides.

Pharmacokinetic Properties

The sulfonamides constitute a series of weak organic acids with pK_a values ranging from 10.4 for sulfanilamides to 5.0 for sulfoxazole. They exist predominantly in the nonionized form in biologic fluids of pH lower than their pK_a . It is the nonionized moiety that diffuses through cell membranes and penetrates cellular barriers.

Table 16.1. Activity of sulfonamides, trimethoprim, and trimethoprim-sulfamethoxazole ($\mu\text{g/ml}$) against selected bacteria.

Organism	Sulfonamide ^a MIC ₉₀	Trimethoprim MIC ₉₀	Trimethoprim- Sulfamethoxazole MIC ₉₀ ^b
Gram-positive aerobes			
<i>Arcanobacterium pyogenes</i>	32	8	0.13
<i>Corynebacterium pseudotuberculosis</i>			≤0.5
<i>C. renale</i>	>64		
<i>Erysipelothrix rhusiopathiae</i>	8	0.13	0.06
<i>Listeria monocytogenes</i>	8	0.06	0.03
<i>Nocardia asteroides</i>	128	128	8
<i>Rhodococcus equi</i>	>128	64	32
<i>Staphylococcus aureus</i>	32	2	0.25
<i>Streptococcus agalactiae</i>	32	0.5	0.06
<i>S. dysgalactiae</i>	>256	4	0.06
<i>S. uberis</i>	>128	4	0.5
Beta-hemolytic streptococci	>128	2	2
Gram-positive anaerobes			
<i>Clostridium perfringens</i>	16	64	
Gram-negative aerobes			
<i>Actinobacillus</i> spp.	64		≤0.06
<i>A. pleuropneumoniae</i> ^c	≥128	2	8
<i>Bordetella bronchiseptica</i> ^c	>256		≤0.06
<i>Brucella abortus</i>	16	4	0.06
<i>B. canis</i>	2		
<i>Campylobacter jejuni</i>	≥256	≥512	≥512
<i>Escherichia coli</i>	≥128	1	≤0.5
<i>Histophilus somni</i>	≥128		
<i>Klebsiella pneumoniae</i> ^c	≥128	4	≤0.5
<i>Moraxella bovis</i>	>64	>64	< 0.15
<i>Pasteurella multocida</i>	>128	4	
<i>Proteus</i> spp.	>256	8	≤0.5
<i>Pseudomonas aeruginosa</i>	>515	512	128
<i>Salmonella</i> spp.	128	4	0.5
<i>Tylorella equigenitalis</i>	>128		
<i>Yersinia enterocolitica</i>	>128	1	8

^aMainly sulfadimethoxine.^bSingle figures refer to trimethoprim concentration; trimethoprim-sulfonamide ratio is 1:19.^cMany of these isolates are now reported as resistant to the combination; this table is partly designed to illustrate the synergism which can occur between sulfonamides and trimethoprim.

Most sulfonamides are rapidly absorbed from the gastrointestinal tract and distribute widely to all tissues and body fluids, including synovial and cerebrospinal fluids. The sulfonamides are bound to plasma proteins to an extent varying from 15 to 90%. In addition, there is variation among species in binding of individual sulfonamides. Extensive (>80%) protein binding increases half-life. In any one species, the extent of protein binding, apparent volume of distribution, and half-life vary widely among individual sulfonamides. This information, together with designat-

ing 100 $\mu\text{g/ml}$ as the desired steady-state plasma sulfonamide concentration, facilitates calculation of dosages.

Sulfonamides are eliminated by a combination of renal excretion and biotransformation. This combination contributes to species variations in the half-lives of individual drugs. Sulfadimethoxine, for example, has half-lives of 12.5 hours in cattle, 8.6 hours in goats, 11.3 hours in horses, 15.5 hours in swine, 13.2 hours in dogs, and 10.2 hours in cats. These relatively long half-lives have been attributed to extensive binding to

plasma albumin and pH-dependent passive reabsorption of the drug from acidic distal renal tubule fluid.

Sulfonamides undergo metabolic alterations to a variable extent in the tissues, especially the liver. Acetylation (which is the principal metabolic pathway for most sulfonamides), glucuronide conjugation, and aromatic hydroxylation take place in humans and in all domestic animals except dogs. It appears that dogs cannot acetylate aromatic amines. Acetylation occurs in the reticulo-endothelial rather than the parenchymal cells of the liver and other tissues such as the lungs. This metabolic reaction has clinical significance, since the acetyl derivative of most sulfonamides (except sulfapyrimidines) has lower aqueous solubility than the parent compound. Acetylation therefore increases the risk of damage to the renal tubules due to precipitation. Aromatic hydroxylation, which may be the principal metabolic pathway for sulfonamides in ruminants, and glucuronide conjugation are microsomal-mediated metabolic reactions. The glucuronide conjugates are highly water-soluble and are rapidly excreted.

Renal excretion mechanisms include glomerular filtration of free (unbound) drug in the plasma, active carrier-mediated proximal tubular excretion of ionized unchanged drug and metabolites, and passive reabsorption of nonionized drug from distal tubular fluid. The extent of reabsorption is determined by the pK_a of the sulfonamide and the pH of the fluid in the distal tubules. Urinary alkalinization increases both the fraction of the dose that is eliminated by renal excretion (unchanged in urine) and the solubility of sulfonamides in the urine.

Drug Interactions

The important synergistic interaction of sulfonamides with antibacterial diaminopyrimidines such as trimethoprim and baquiloprim is discussed under Diaminopyrimidines below.

The agents appear not to antagonize the bactericidal effect of penicillins, but the procaine of procaine penicillin is an analog of PABA that will antagonize sulfonamides. Combination with pyrimethamine is the treatment of choice for toxoplasmosis and some other protozoal infections.

Toxicity and Adverse Effects

The sulfonamides can produce a wide variety of usually reversible side effects; some may have an allergic

basis and others are the result of direct toxicity. The more common adverse effects are urinary tract disturbances (crystalluria, hematuria, or even obstruction), hematopoietic disorders (thrombocytopenia, anemia, leukopenia), and dermatologic reactions. Significant reactions, however, are generally uncommon in animals treated with conventional doses of common sulfonamides (other than sulfaquinoxaline) for less than 2 weeks.

In a small proportion (approximately 0.25%) of humans or animals, sulfonamide therapy can produce idiosyncratic drug reactions, which are unpredictable and rare events occurring 10 days to 3 weeks after first exposure. The syndrome in dogs includes fever, arthropathy, blood dyscrasias, epistaxis, hepatopathy, skin eruptions of various types, uveitis, and keratoconjunctivitis sicca (Noli et al., 1995; Trepanier, 2004). These are sometimes described as hypersensitivity reactions (drug fever, urticaria), since they seem to involve immune reactions such as a T-cell-mediated response to proteins haptenated by sulfonamide metabolites (Trepanier, 2004). Alternatively, they may involve a limited capacity to detoxify metabolites of sulfonamides (Cribb and Spielberg, 1990). Idiosyncratic reactions recur if individuals are retreated with sulfonamides. In dogs, serious but reversible sulfadiazine-induced reactions have been described in a number of reports on Doberman Pinschers, in which sulfonamides should probably be avoided.

Some adverse effects are associated with particular sulfonamides. Sulfadiazine and sulfasalazine given for long periods to dogs as a "geriatric stimulant" have caused keratoconjunctivitis sicca (KCS), which was not always fully reversible when the drug was discontinued. However, in one study KCS determined by decreased tear production occurred in 15% of 33 dogs treated with trimethoprim-sulfadiazine combination, within the first week of treatment (Berger et al., 1995). This effect occurred in dogs weighing less than 12 kg, suggesting that dosage must be particularly carefully calculated for small dogs.

Renal tubular damage can be minimized by ensuring that the patient is well-hydrated throughout the course of treatment, by administering the most soluble sulfonamides, and by alkalinizing the urine. Prolonged dosage with sulfa-ethoxypyridine in dogs has produced cataracts. Sulfaquinoxaline has caused hypothermia, hemorrhage, and death in puppies given the drug orally for control of coccidiosis; hemorrhagic

Table 16.2. Examples of usual dosages of sulfonamides in animals.

Drug	Route	Dose (mg/kg)	Dosing Interval (hrs)	Comment
Short-acting: sulfadiazine, sulfamethazine, trisulfapyrimidine (triple sulfa)	IV, PO	50-60	12	Double first dose
Sulfamethoxazole	PO	50	12	Double first dose
Intermediate-acting:				
Sulfadimethoxine (SDM)	PO, IV, IM, SC	27.5	24	Double first dose
SDM sustained release, cattle	PO	137.5	96	
Sulfadiazine	PO, IV	50	12	Double first dose
Sulfisoxazole	PO	50	8	Urinary tract infections
Gut-active: Phthalylsulfathiazole	PO	100	12	
Special-use:				
Salicylazosulfapyridine	PO	25	12	See text
Silver sulfadiazine	Topical			

diathesis was reported in other species because of the antagonistic effect of this drug on vitamin K.

Rare additional adverse effects reported include: hepatic necrosis leading to death or euthanasia, developing in some cases within days of treatment (Twedt et al., 1997), and hypothyroidism associated with prolonged treatment (Torres et al., 1996). An unusual goitrogenic effect in swine, which increased the number of stillborn or weak piglets born to sows fed sulfadimethoxine and ormetoprim in late gestation, was described by Blackwell et al. (1989). Congenital defects have been described in foals born to mares treated for equine protozoal myeloencephalitis during pregnancy (Toribio et al., 1998).

Administration and Dosage

In treating systemic diseases with sulfonamides, it is desirable to initiate therapy with a priming dose and to administer maintenance doses, each one-half the priming dose, at intervals approximately equal to the half-life of the drug (Table 16.2). When the drug is administered orally, the dose level must compensate for incomplete systemic availability from the oral preparation, ie % bioavailability of oral preparations.

While a large number of sulfonamide preparations are available for use in veterinary medicine, many of these are different dosage forms of sulfamethazine. This sulfonamide is most widely used in food-producing animals and can attain effective plasma concentrations when administered either orally or parenterally. Because of their alkalinity, most parenteral preparations should be administered only by IV

injection. Rapid IV injection of high doses of sulfonamide preparations should be avoided. Sulfamethazine therapy should be initiated with an IV priming dose of 100 mg/kg, and effective concentrations can then be maintained by administering maintenance doses of 50 mg/kg PO at 12-hour intervals. At least one prolonged-release oral preparation of sulfamethazine is available for use in calves and could be administered to sheep and goats. This is a convenient form of maintenance therapy in that a single dose provides an effective level for 36–48 hours. Different oral forms have different systemic availability (Table 16.3).

Sulfadimethoxine preparations are more widely used in companion animals. The parenteral preparation (40%), containing sulfadimethoxine sodium in solution, is suitable for IV administration to horses. Having initiated therapy with a priming dose of 50 mg/kg, effective concentrations can be maintained with maintenance dosage of 25 mg/kg IV at 12-hour intervals. In dogs and cats, sulfadimethoxine can be administered either as the parenteral solution IV or as the oral suspension. Therapy should be initiated with a priming dose (55 mg/kg IV), and therapeutic concentrations can be maintained by administering maintenance doses IV (27.5 mg/kg) or PO (55 mg/kg) once daily or divided over 12-hour intervals. Selection of the dosing interval should be based on quantitative susceptibility of the pathogenic microorganisms and the site of infection.

Sulfisoxazole has higher aqueous solubility than most other members of the class. Its solubility in urine increases markedly with increase in urinary pH. It has

a half-life in dogs of 4.5 hours, and because it is eliminated largely by renal excretion, sulfisoxazole is present in high concentrations unchanged in the urine. This makes sulfisoxazole an effective agent in the treatment of urinary tract infections caused by susceptible organisms. The usual oral dosage is 50 mg/kg administered at 8-hour intervals.

Unlike the sodium salts of other sulfonamides, sodium sulfacetamide is nearly neutral. It is the only sulfonamide available for topical ophthalmic use. A 30% solution applied to the conjunctivae penetrates well and attains high concentrations in ocular fluids and tissues.

Clinical Applications

Widespread resistance greatly limits the effectiveness of sulfonamides in treating bacterial diseases of animals, so that indications for primary use are few. Trimethoprim- or other antibacterial diaminopyrimidine-sulfonamide combinations have largely replaced sulfonamides alone as therapeutic agents in companion animals, although resistance also increasingly limits these combinations' use. Purulent material must always be removed, since free purines neutralize the effect of sulfonamides. Primary uses include treatment of toxoplasmosis (when combined with pyrimethamine), chlamydiosis, *Pneumocystis carinii*, and possibly nocardiosis (combined with minocycline), and the use of sulfasalazine in the treatment of chronic colitis.

Cattle, Sheep, and Goats

Widespread resistance limits the use of sulfonamides in these animals, and it is best to give these agents in combination with trimethoprim. Orally administered, long-acting, sustained-release dosage forms result in effective plasma concentrations for 3–5 days. Such a preparation has been effective in clinical trials assessing prevention and treatment of feedlot pneumonia, an unexpected result in view of the resistance reported in bovine *Pasteurella*. Sulfonamides are used successfully to treat bovine interdigital necrobacillosis and coccidiosis. Sulfadimethoxine is the only sulfonamide approved for use in dairy cows over 20 months of age in the United States; extra-label use in dairy cows is prohibited. Sustained-release oral sulfamethazine and orally administered pyrimethamine, 0.5 mg/kg once daily, might be drugs of choice in preventing outbreaks of *Toxoplasma* abortion in sheep. Sulfonamides have been used with chlortetracyclines in feedlot

lambs to improve performance and prevent clostridial enterotoxemias.

Swine

Sulfonamides have been used to promote growth and to control group E streptococcal infections and atrophic rhinitis in swine. The sulfonamides are often combined with chlortetracycline. In the United States, there have been moves to ban the use of sulfonamides for use in swine because of persistent problems of residues in carcasses in excess of legally permitted concentrations and evidence from chronic toxicity studies in mice that sulfamethazine was linked to the production of thyroid adenomas in rodents.

Horses

Sulfonamides are used in horses in combination with antibacterial diaminopyrimidines. For the treatment of equine protozoal myeloencephalitis, sulfadiazine (20 mg/kg PO SID or BID, for up to 12 weeks or longer) combined with pyrimethamine (1.0 mg/kg PO SID, for up to 120 days or longer) (Dubey et al., 2001). Dapsone alone (3 mg/kg PO SID) has been used successfully in the treatment of *Pneumocystis carinii* pneumonia in a foal (Clark-Price et al., 2004).

Dogs and Cats

Use of sulfisoxazole to treat urinary tract infections in dogs has been largely replaced by antibiotics that are more effective because of their broader spectrum of activity or bactericidal action. Sulfonamides are one of the drugs of choice in the treatment of *Nocardia* infections; effectiveness may be increased by concurrent administration of minocycline (Chapter 23). Silver sulfadiazine cream has been used as a treatment in chronic otitis externa caused by multi-resistant *P. aeruginosa*, as the drug acts as a broad-spectrum antimicrobial antiseptic. This preparation has been effective in controlling bacteria that infect burn wounds in human patients.

Sulfasalazine (salicylsulfapyridine) has been recommended as a drug of choice in the treatment of chronic colitis in dogs. It is hydrolyzed by colonic bacteria to yield sulfapyridine and 5-aminosalicylate; it is likely that the anti-inflammatory effect of the latter is responsible for the therapeutic effect. Comparably high concentrations of salicylate cannot be achieved in the colon by oral administration. The dosage of sulfasalazine for the dog is 25 mg/kg PO 3 times daily.

The same dose in cats may induce salicylate poisoning (Burrows, 1983). Some have suggested that a low dose of corticosteroid be administered simultaneously to reduce the overall duration of therapy, which is 3–4 weeks when the drug is administered alone. This dual dosage may decrease the frequency of keratoconjunctivitis sicca. In most cases of sulfasalazine treatment, cure is achieved within 4 weeks, and treatment should not be continued beyond this time without histologic confirmation of colonic inflammation.

Dapsone (diaminodiphenylsulphone) has been used in the treatment of dermatitis herpetiformis in dogs and in the treatment of leprosy in humans.

Poultry

Sulfonamides have been used in the prevention and treatment of coccidiosis, infectious coryza, pullorum disease, and fowl typhoid.

Bibliography

- Berger SL, et al. 1995. A quantitative study of the effects of Tribissen on canine tear production. *J Am Anim Hosp Assoc* 31:236.
- Blackwell TE, et al. 1989. Goitrogenic effects in offspring of swine fed sulfadimethoxine and ormetoprim in late gestation. *J Am Vet Med Assoc* 194:519.
- Burrows CE. 1983. The treatment of diarrhea. In: Kirk RW (ed). *Current Veterinary Therapy VIII: Small Animal Practice*. Philadelphia: WB Saunders.
- Clark-Price SC, et al. 2004. Use of dapsone in the treatment of *Pneumocystis carinii* pneumonia in a foal. *J Am Vet Med Assoc* 224:407.
- Cribb AE, Spielberg SP. 1990. An in vitro investigation of predisposition to sulphonamide idiosyncratic toxicity in dogs. *Vet Res Comm* 14:241.
- Dubey JP, et al. 2001. A review of *Sarcocystis neurona* and equine protozoal myeloencephalitis (EPM). *Vet. Parasitol.* 95:89.
- Lanz R, et al. 2003. Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animals species in Switzerland. *Vet Microbiol* 91:73.
- Maynard C, et al. 2003. Antimicrobial resistance genes in enterotoxigenic *Escherichia coli* O149:K91 isolates obtained over a 23-year period from pigs. *Antimicrob Agents Chemother* 47:3214.
- Noli C, et al. 1995. A retrospective evaluation of adverse reactions to trimethoprim-sulphonamide combinations in dogs and cats. *Vet Quart* 17:123.
- Trepanier LA. 2004. Idiosyncratic toxicity associated with potentiated sulfonamides in the dog. *J Vet Pharm Therap* 27:129.
- Toribio RE, et al. 1998. Congenital defects in newborn foals of mares treated for equine protozoal myeloencephalitis during pregnancy. *J Am Vet Med Assoc* 212:697.
- Torres SMF, et al. 1996. Hypothyroidism in a dog associated with trimethoprim-sulphadiazine therapy. *Vet Dermatol* 7:105.
- Twedt DC, et al. 1997. Association of hepatic necrosis with trimethoprim-sulfonamide administration in 4 dogs. *J Vet Intern Med* 11:20.
- Weiss DJ, Klansner JS. 1990. Drug-associated aplastic anemia in dogs: eight cases (1984–1988). *J Am Vet Med Assoc* 196:472.
- Wilson RC, et al. 1989. Bioavailability and pharmacokinetics of sulfamethazine in the pony. *J Vet Pharm Ther* 12:99.

Antibacterial Diaminopyrimidines: Aditoprim, Baquiloprim, Ormetoprim and Trimethoprim

Diaminopyrimidines interfere with folic acid production by inhibition of dihydrofolate reductase. Some diaminopyrimidines have marked specificity for bacterial dihydrofolate reductases (aditoprim, baquiloprim, ormetoprim, trimethoprim), others for protozoal enzymes (pyrimethamine), and others for mammalian enzymes (methytrexate). The earliest antibacterial diaminopyrimidine introduced for clinical use was trimethoprim (Figure 16.2), a synthetic drug that is widely used in combination with sulfonamides. It is a weak base with a pK_a of about 7.6 and is poorly soluble in water. Other antibacterial diaminopyrimidines have similar antibacterial activities to trimethoprim but offer significant pharmacokinetic advantages, particularly those with greater half-lives and tissue distribution.

Mechanism of Action

Diaminopyrimidines interfere with the synthesis of tetrahydrofolic acid from dihydrofolate by combining with the enzyme dihydrofolate reductase. Selective antibacterial activity occurs because of greater affinity for the bacterial rather than the mammalian enzyme. Diaminopyrimidines thus inhibit the same metabolic sequence as the sulfonamides, preventing bacterial synthesis of purines and thus of DNA. A synergistic and bactericidal effect occurs when the diaminopyrimidines are combined with sulfonamides (see sulfonamide-diaminopyrimidine combinations), and for this reason these drugs are invariably used with a sulfonamide in veterinary medicine.

Interestingly, in the United Kingdom trimethoprim alone rather than the combination is now generally

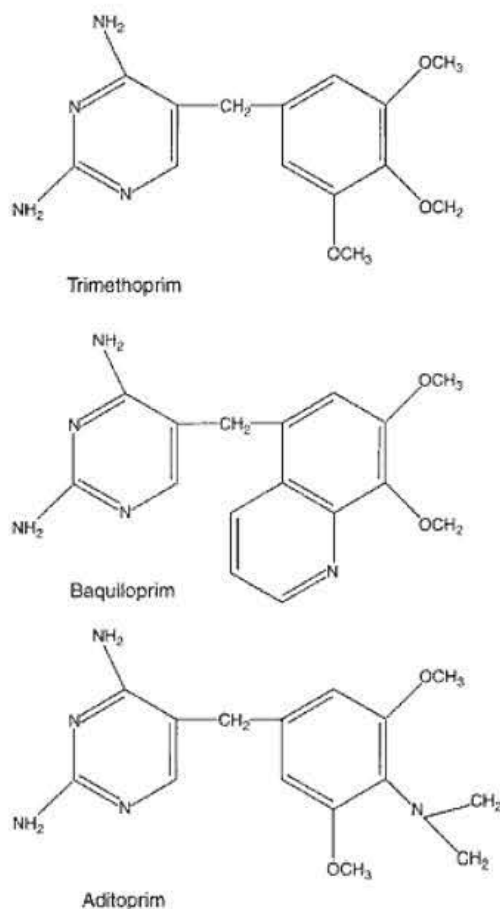


Figure 16.2. Structural formulas of some diaminopyrimidines.

used in human medicine (Hughes, 1997). The reasons for the abandonment of the trimethoprim-sulfonamide combination in favor of trimethoprim alone are (1) bacteriostatic synergy is only demonstrable when the concentration of each drug is less than bacteriostatic, but the bacteriostatic effect of trimethoprim in urinary tract infections, for which the drug is most commonly used, is often detectable in urine for several days; (2) diaminopyrimidines are more widely distributed into tissues than sulfonamides, reaching sites, such as cells, which sulfonamides do not penetrate well; (3), most of the adverse effects of the combination are the result of the sulfonamide component; and, (4) the original claim that the combination prevented the emergence of resistance is dubious because sulfonamide resistance is widespread and because plasmids conferring resistance to sulfonamides often also confer resistance to trimethoprim (Hughes, 1997). The licensed medical use in the United King-

dom of the combination is therefore restricted largely to the treatment of *Pneumocystis carinii* infection.

Antimicrobial Activity

Antibacterial diaminopyrimidines are generally bacteriostatic, broad-spectrum drugs active against Gram-positive and Gram-negative aerobic bacteria, but not usually against anaerobes (Table 16.1). Bacteria with an MIC ≤ 1 $\mu\text{g/ml}$ are usually regarded as susceptible. Activity against *Mycoplasma* spp., *Chlamydia* spp., *Mycobacterium* spp., and *P. aeruginosa* is negligible. Activity of aditoprim, baquilogrim, and ormetoprim is similar to or very slightly less than that of trimethoprim.

Resistance

Resistance to trimethoprim and other diaminopyrimidines is usually the result of transposon- or integron-encoded plasmid or chromosomal synthesis of a resistant dihydrofolate reductase enzyme (Skold, 2001). Resistance is increasingly reported, particularly among Enterobacteriaceae. About 20 phylogenetically different resistance genes expressing dihydrofolate reductases have been characterized. Isolates with plasmid- or integron-mediated resistance commonly show multiple resistances, including sulfonamide resistance. Examples include multi-drug-resistant *Salmonella* such as *S. typhimurium* DT104 and *S. newport*. The apparent spread of a trimethoprim resistance gene from porcine to human *E. coli* has been described (Jansson et al., 1992).

Pharmacokinetic Properties

Diaminopyrimidines including trimethoprim are lipid-soluble organic bases that are approximately 60% bound to plasma proteins. The drugs distribute widely, penetrating cellular barriers by nonionic diffusion and attaining effective concentrations in most body tissues and fluids. The drug may concentrate in fluids, such as those found in the prostate, that are acidic relative to plasma. The average milk-to-plasma equilibrium concentration ratio is 3:1. The dose, systemic availability from the dosage form, and route of administration determine the plasma concentration profile and tissue levels of the drug. Hepatic metabolism (oxidation followed by conjugation reactions) is the principal process for elimination. Because of this, the half-life and fraction of the dose that is excreted unchanged in the urine vary widely among species. In ruminants, the

short half-life of trimethoprim is due to rapid demethylation to produce inactive compounds. Replacing the phenyl ring of trimethoprim with the bicyclic ring of baquiloprim resulted in an increase in half-life from 1 hour (trimethoprim) to 10 hours (baquiloprim) in cattle and from about 2 to 5 hours in pigs, while replacement of a methyl group in trimethoprim by the dimethylamino group of aditoprim increased its half-life in cattle to 4–7 hours, in horses to 9–14 hours, and in pigs to 8–9 hours, or greater. Greater tissue distribution may be one factor responsible for prolonged half-life compared to trimethoprim.

Toxicity and Adverse Effects

The antibacterial diaminopyrimidines are relatively nontoxic drugs. Their main, though clinically unimportant potential toxic effect, is to induce folic acid deficiency at high doses. Rarely, aseptic meningitis related to trimethoprim therapy has been reported in humans. Hyperkalemia may occur under unusual circumstances (Rubin et al., 1993).

Clinical Applications

Antibacterial diaminopyrimidines are currently used only in combination with sulfonamides in animals, although there may be a need to reassess the benefits of the combination. Alone or in combination they may be a drug of choice for treating prostatic infections caused by Gram-negative bacteria, since prostatic concentrations may reach 10 times those of plasma, at which concentration the drug may be bactericidal. Nevertheless, clinical results in treating chronic prostatitis with trimethoprim may be disappointing, probably because of the nature of the disease process. Trimethoprim administered orally has been used to prevent relapse after treatment of *L. monocytogenes* meningitis in humans. Antibacterial diaminopyrimidines, including trimethoprim, combined with sulfonamides or dapsone may be the prophylactic drugs of choice for *Pneumocystis carinii* pneumonia (Hughes, 1988).

Bibliography

- Brown MP, et al. 1989. Pharmacokinetics and body fluid and endometrial concentrations of ormetoprim-sulfadimethoxine in mares. *Can J Vet Res* 53:12.
- Davies AM, MacKenzie NM. 1994. Pharmacokinetics of baquiloprim and sulphadimidine in pigs after intramuscular injection. *Res Vet Sci* 57:69.

- Hughes WT. 1988. Comparison of dosages, intervals, and drugs in the prevention of *Pneumocystis carinii* pneumonia. *Antimicrob Agents Chemother* 32:623.
- Jansson C, et al. 1992. Spread of a newly found trimethoprim resistance gene, *dhfrIX*, among porcine isolates and human pathogens. *Antimicrob Agents Chemother* 36:2704.
- Rubin SI, et al. 1998. Trimethoprim-induced exacerbation of hyperkalemia in a dog with hypoadrenocorticism. *J Vet Intern Med* 12:186.
- Skold O. 2001. Resistance to trimethoprim and sulfonamides. *Vet Res* 32:261.
- Van Miert ASJPAM. 1994. The sulfonamide-diaminopyrimidine story. *J Vet Pharm Ther* 17:309.
- West B, White G. 1979. A survey of trimethoprim resistance in the enteric bacterial flora of farm animals. *J Hyg* 82:481.

Antibacterial Diaminopyrimidine-sulfonamide Combinations

Antibacterial diaminopyrimidines are combined with a variety of sulfonamides (sulfadiazine, sulfamethoxazole, and sulfadoxine) in a fixed (1:5) ratio, which in people produces a 1:20 ratio of drug concentrations in the plasma after oral or parenteral administration. This ratio is desirable since maximum synergy occurs when the drugs are present in the ratio of their MICs; diaminopyrimidines are 20–100 times more active than the sulfonamides, so that combinations are formulated to give a 1:20 ratio in human serum. This ratio occurs because diaminopyrimidines (lipid-soluble organic bases) are concentrated in tissues, whereas sulfonamides (weak organic acids) remain largely in extracellular fluids. At these MICs and in this ratio, the combination produces a bactericidal effect against a wide range of bacteria, with some important exceptions, and also inhibits certain other microorganisms. Since the combinations of different diaminopyrimidines with sulfonamides give essentially similar antibacterial effects, comments will relate largely to trimethoprim-sulfonamide combinations but can be extrapolated to other combinations.

Veterinary preparations follow medical usage in that they contain diaminopyrimidines combined with a sulfonamide in the 1:5 ratio. For trimethoprim, the half-lives of the components (sulfadiazine, sulfadoxine, or sulfamethoxazole) do not coincide in any species (except humans) whereas they are more similar for baquiloprim (sulfadimidine, sulfadimethoxine) and ormetoprim (sulfadimethoxine). The dosage aims at maintaining bacteriostatic concentrations of the

sulfonamide, which, for a time after each dose, is enhanced by the synergistic bactericidal action of the combination.

Mechanism of Action

The combination of a diaminopyrimidine with a sulfonamide inhibits sequential steps in the synthesis of folic acid and thus of the purines required for DNA synthesis. The interference by the diaminopyrimidine trimethoprim with recycling of tetrahydrofolic or dihydrofolic acid is probably responsible for the synergistic interaction of the combination.

Antimicrobial Activity

Diaminopyrimidine-sulfonamide combinations have a generally broad and usually bactericidal action against many Gram-positive and Gram-negative aerobic bacteria, including Enterobacteriaceae, *Actinomyces*, *Nocardia*, *Chlamydia*, and protozoa such as *Toxoplasma*. They are not active against anaerobic bacteria in vivo because thymidine and PABA in the necrotic tissue antagonize their antibacterial effect. Such an antagonistic effect is not limited to anaerobes, so that this combination may not be fully effective in closed, non-draining infections where there is significant tissue debris. *Pneumocystis carinii* and some malarial parasites are susceptible; *Mycoplasma* and *P. aeruginosa* are resistant.

Synergism occurs when the microorganisms are susceptible to both drugs in the combination. It may still be obtained, in up to 40% of cases, when bacteria are resistant to sulfonamides. Synergy often occurs if the organism is resistant to trimethoprim but sensitive to sulfonamides and in nearly 40% of cases in which the organism is resistant to each drug alone. Nevertheless, many organisms described as susceptible to the combination are susceptible to the diaminopyrimidine component only. Clinical response may sometimes be lower than expected from in vitro data, and better understanding of the use of MIC data in prediction of clinical outcome is required. One element of such disappointing responses may also be the presence of thymidine and PABA in infected tissue. A more important element may be widespread resistance to sulfonamides and consequently the lack of synergism in many cases, so that only the diaminopyrimidine component is active. For trimethoprim, the short half-life in some species may exacerbate a lack of synergism.

Where synergistic interactions occur, a 10-fold increase in activity of the trimethoprim component and a 100-fold increase in activity of the sulfonamide component are common. Synergism occurs at different drug concentration ratios with different bacterial species. Because of differences between the diaminopyrimidine and sulfonamide in distribution and, in the case of trimethoprim, of elimination, the concentration ratios may differ considerably in tissues and urine from that in plasma. Such variation is said not to be important, as the synergistic interaction may occur over a wide range of concentration ratios of the drugs—but clearly it would not occur in some tissues, since diaminopyrimidines are distributed more widely than sulfonamides. Because of these variations in the pharmacokinetics of diaminopyrimidines and sulfonamides, the length of effective action is difficult to assess based on serum concentrations alone. This has given rise to the suspicion that the manufacturer's recommended dosages are less than optimal, especially for trimethoprim combinations. A number of recent pharmacokinetic studies have resulted in recommendations to increase dosage (Ensink et al., 2003, 2005).

Errors in laboratory testing are common because of the presence of PABA or thymidine in media; in one study, half the strains reported as resistant in other laboratories were susceptible when tested in a reference laboratory. The use of lysed horse blood, which contains thymidine phosphorylase, will eliminate excess thymidine in culture media.

Good susceptibility (MIC trimethoprim/sulfonamide $\leq 5/9.5$ $\mu\text{g/ml}$) is shown among the following Gram-positive aerobes: *S. aureus*, streptococci, *Actinomyces* spp., *Corynebacterium* spp., *E. rhusiopathiae*, and *L. monocytogenes*. Gram-negative aerobes: *Actinobacillus* spp., *Bordetella* spp., *Brucella* spp., Enterobacteriaceae such as *E. coli*, *Klebsiella* spp., *Proteus* spp., *Salmonella* spp., *Yersinia* spp., *Haemophilus* spp., and *Pasteurella* spp. Anaerobes: *Actinomyces* spp., *Bacteroides* spp., *Fusobacterium* spp., some *Clostridium* spp., and *Chlamydia* spp.

Moderate activity (MIC $\leq 2/38$ $\mu\text{g/ml}$) includes some *Mycobacterium* spp. and some *Nocardia* spp.

Resistance (MIC $\geq 4/76$ $\mu\text{g/ml}$) is shown by *Rickettsia*, *Leptospira* spp., *P. aeruginosa*, and *Mycoplasma* spp. (Table 16.1).

Resistance

Mechanisms of resistance were discussed under the individual components of the combination. Resistance to the combination has developed progressively with use. Multiple integron-associated resistance, which includes both sulfonamide and trimethoprim resistance, has been described in some *Salmonella* serovars and in pathogenic *E. coli* isolated from animals.

Pharmacokinetic Properties

In humans the half-lives of trimethoprim and sulfamethoxazole are similar, and maintenance dosage provides continuous, therapeutic concentrations of both drugs in plasma. In animals the half-lives of the drugs are not similar, but the combination is often clinically effective because of the relatively broad range of drug ratios over which synergism occurs. For reasons discussed earlier, the diaminopyrimidine component concentrates in tissues, whereas the sulfonamide component moves only slowly from plasma into tissues. The longer half-lives of newer diaminopyrimidines (baquilogrim, ormetoprim) give the advantages of better maintenance of the 1:20 ratio said to be desirable, and of less frequent dosing.

Following SC injection in cattle, trimethoprim seems to deposit in a slow-release form, so that serum concentrations remain below MIC. Because of this, the SC route cannot be recommended in cattle and perhaps in other species.

Drug Interactions

Trimethoprim-sulfonamide has sometimes been used in conjunction with ampicillin to provide "broad-spectrum bactericidal antimicrobial coverage" before microbiology data are available. However, one study showed that addition of ampicillin to trimethoprim-sulfonamide dosing regimens only marginally increased the spectrum of activity. There is no known mechanism to suggest that such a combination might be synergistic. Rather, such a combination may be effective in treating polymicrobial infections involving aerobic bacteria susceptible to the trimethoprim-sulfonamide combination and anaerobic bacteria susceptible to ampicillin.

Toxicity and Adverse Effects

The combination has a wide margin of safety, and adverse effects can mainly be attributed to the sulfonamide. These effects are discussed in the general description of the adverse effects of each drug class.

In horses, minor tissue damage and pain may occur after IM injection; transient pruritus has been reported to follow the first but not subsequent doses. In isolated incidents, a fatal adverse reaction (possibly respiratory failure) followed IV injection of the combination in horses (in some cases in anesthetized horses). A 7% incidence of diarrhea was observed in a study of the effect of twice-daily administration of oral 30 mg/kg trimethoprim-sulfadiazine in horses. The prevalence of diarrhea noted following trimethoprim-sulfonamide use in horses in another study was not significantly different from that observed in horses receiving other antibiotics, including penicillin (Wilson et al., 1996).

Administration and Dosage

Usual dosages are shown in Table 16.3. Dogs and cats can be given the oral form (tablets) at the same dosage. Twice daily oral dosing of horses with 30 mg/kg trimethoprim-sulfadiazine combination paste, rather than once daily, is recommended. In mares, oral dosage with ormetoprim-sulfadimethoxine paste recommended for susceptible organisms was a loading dose of 9.2 mg ormetoprim and 45.8 mg sulfadimethoxine/kg followed by half this dose every 24 hours (Brown et al., 1989).

Clinical Applications

Diaminopyrimidine-sulfonamide combinations have the advantage of good distribution into tissues, safety, a relatively broad-spectrum bactericidal activity, and oral administration. A disadvantage is antagonism of action by infected tissue debris.

The combination can be recommended in the treatment of urinary tract infections caused by common opportunist pathogens. The combination has a particular

Table 16.3. Usual dosages of potentiated sulfonamide combinations in animals.

Drug (species)	Route Comments	Dose (mg/kg)	Dosing Interval (hrs)
Trimethoprim-sulfonamide	PO, IV, IM Not IM in horses	15 or 30	12 or 24
Ormetoprim-sulfadimethoxine	PO Double first dose	27.5	24
Baquilogrim-sulfadimethoxine			
Dogs	PO	30	48
Cats	PO	30	24
Cattle, swine	IM	10	24

place in the treatment of bacterial prostatitis because of good tissue penetration. Other indications include the treatment of enteric infections (*E. coli*, *Salmonella*, *Yersinia enterocolitica*). The drug is of value in the treatment of brucellosis, often in combination with rifampin or doxycycline. The combination is a drug of choice in the treatment of *Nocardia* infections, but high oral dosage (3 mg trimethoprim equivalent/kg every 6 hours) must be used for prolonged periods.

Other indications include the treatment of *Pneumocystis carinii*, *Chlamydia* infections, certain mycobacterial infections (*M. kansasii*, *M. marinum*), and *Coxiella* infections. The drug is also used in the treatment of acute upper and lower respiratory tract infections caused by susceptible organisms.

Cattle, Sheep, and Goats

The drug combination is widely used in dairy and beef cattle and has been used successfully in the treatment of salmonellosis in calves, as well as in undifferentiated diarrhea, bacterial pneumonia, foot rot, and septicemic colibacillosis. Baquiloprim-sulfadimidine was not as efficacious as danofloxacin in the treatment of experimentally induced *E. coli* diarrhea in calves (White et al., 1998), presumably because the organism is less susceptible to the combination drug. The potential for use in coliform septicemia and meningitis seems excellent but may be limited by resistance. In meningitis the drug should be administered IV 3 or 4 times daily at the usual dosage. The potential for use in the treatment of *Listeria* meningoencephalitis appears excellent. The susceptibility of *Histophilus somni*, *Pasteurella multocida*, some *Mannheimia haemolytica*, and *Arcanobacterium pyogenes* suggests a useful application in bovine respiratory disease; this has been borne out by field studies. The drug combination should be administered parenterally (not orally). Clinical trials with undifferentiated bovine respiratory disease have failed to demonstrate improvement when dosage of trimethoprim-sulfadoxine was increased beyond that recommended or when the product was administered IV compared to IM, although pharmacokinetic studies suggest that the manufacturer's once-daily recommended dosage of 16 mg/kg is too low. A preferred minimum dosage is 30 mg/kg SID or 15 mg/kg BID. Experimental studies have shown the antagonistic effect of infected tissue debris on the action of the combination (Greko et al., 2002).

When used to treat acute mastitis, the drug should

be given IV at high dose because of poor bioavailability after IM injection and relatively poor udder penetration; a dosage of 48–50 mg/kg every 12 hours is appropriate for acute mastitis. A beneficial effect of trimethoprim-sulfonamide on the treatment of coliform mastitis has been noted, particularly when combined with non-steroidal anti-inflammatory drugs (Shpigel et al., 1998).

Other uses in cattle include the treatment of urinary tract infections and mixed aerobe-anaerobe infections, such as those occurring in post-parturient metritis. The drug has potential but unproven use for the treatment of *L. monocytogenes* encephalitis in ruminants.

A special application in sheep is in preventing *Toxoplasma* abortion; the drug is also potentially useful in preventing chlamydial abortion. In experimental *Toxoplasma* infections in mice, protection by trimethoprim-sulfonamide was inferior to pyrimethamine-sulfadiazine, but clinical results in naturally occurring infections in humans have been excellent.

Swine

Trimethoprim-sulfonamide combinations have been used successfully in controlling a wide variety of conditions in pigs, including neonatal and post-weaning colibacillosis, salmonellosis, atrophic rhinitis, greasy pig disease, streptococcal meningitis, and pneumonia. Atrophic rhinitis may be controlled by incorporating the drug in feed or water, or by injecting piglets at various times, (e.g., the third day of life and again in the third and sixth weeks). The mastitis-metritis-agalactia syndrome has been controlled by the prophylactic administration of 15 mg/kg PO for 3 days before and 2 days after parturition. The combination has been used in the eradication of *A. pleuropneumoniae* infection from herds by treating adults through the water for 3 weeks in combination with removal of serologically positive animals. Other diaminopyrimidine-sulfonamide combinations are available for swine for similar purposes to trimethoprim-sulfonamide combinations (Table 16.3).

Horses

The combination of trimethoprim-sulfadiazine is popular in horses because it can be administered orally with few adverse effects. It is painful when administered IM. It is, therefore, used orally to treat acute respiratory infections including strangles, acute urinary tract infections, and wounds and abscesses,

and is a drug of choice in salmonellosis. In recent years, however, resistance has apparently increased in *Streptococcus zooepidemicus*, so that in some studies less than 90% of isolates are susceptible in vitro (Peyrou et al., 2003). The combination is ineffective in eradicating *S. equi* subspecies *zooepidemicus* in a tissue chamber model of infection despite in vitro susceptibility of the isolate and high concentrations of the drugs in the tissue chamber fluid (Ensink et al., 2003). For these reasons, and because it can be partially antagonized by tissue debris, trimethoprim-sulfadiazine is a less desirable choice than procaine penicillin G for treatment of streptococcal infections.

In foals the combination is used in the treatment of *Actinobacillus* and coliform infections, although the latter use may be compromised by resistance. The drug may be used for coliform meningitis, in which high doses should be administered slowly IV 3 or 4 times daily. The drug may otherwise be administered orally, but oral dosage recommended by the manufacturers may be low and there is apparent advantage to twice daily dosage (30 mg/kg) of oral preparations (Van Duijkeren et al., 1994). The combination of sulfadiazine with pyrimethamine is a drug of choice in the treatment of protozoal encephalomyelitis (see Antiprotozoal Diaminopyrimidines). It is a drug of choice for *P. carinii* infections in foals. Direct infusion of the combination into the uterus may cause endometrial inflammation.

Dogs and Cats

Trimethoprim-sulfonamide or ormetoprim-sulfadimethoxine combinations have wide application in dogs and cats against specific and nonspecific infections. The combination is highly effective against many opportunist bacteria present in canine urinary tract, skin, and ear infections (*S. intermedius*, streptococci, and Enterobacteriaceae including *E. coli* and *Proteus*). The drug has the potential for use in prophylaxis of urinary tract infections.

Consideration should be given to twice-daily dosing with trimethoprim-sulfadiazine. A blinded comparison of once- versus twice-daily dosing with 30 mg/kg trimethoprim-sulfadiazine in the treatment of canine pyoderma showed an advantage of twice-daily dosing, although this was not statistically significant, possibly because of small numbers of animals in the trial (Messinger and Beale, 1993). In one study, however, mean serum and skin concentrations using once-daily

dosing were considered to achieve therapeutically effective concentrations (Pohlenz-Zertuche et al., 1992).

The combination drug is effective against *Bordetella bronchiseptica*, although relapses after treatment with trimethoprim-sulfadiazine for 5 days were common in experimental kennel cough. The drug should probably be administered for several weeks in the treatment of this infection. In one study, a significant number of isolates were found to be resistant to the combination drug (Speakman et al., 2000), so that doxycycline or amoxicillin-clavulanic acid may now be a better choice for treatment of kennel cough. The drug has been used successfully in the treatment of canine actinomycosis, often in conjunction with procaine penicillin; the combination may be particularly useful where *Nocardia* and *A. viscosus* have not been distinguished properly. Trimethoprim-sulfadiazine has been effective against coccidiosis in dogs and cats.

Excellent penetration into the prostate makes the combination a treatment of choice in Gram-negative prostatic infections in dogs, equal to or better than minocycline, although now challenged by the fluoroquinolones. Similarly, the excellent penetration (50% of serum concentration) of the aqueous and vitreous humors of the eyes by both drugs makes the combination suitable in the parenteral treatment of panophthalmitis caused by Gram-negative bacteria. Trimethoprim-sulfadiazine is used together with clindamycin and pyrimethamine in the initial treatment of *Hepatozoon* infections in dogs.

Poultry

Trimethoprim-sulfaquinoxaline and sulfamethoxazole-ormetoprim are used in the prophylaxis and treatment of *E. coli*, *Haemophilus*, and *Pasteurella* infections, as well as of coccidiosis. These combinations have been used successfully in the treatment of *Plasmodium galinaceum* malaria in chickens (Williams, 2005).

Bibliography

- Brown MP, et al. 1989. Pharmacokinetics and body fluid and endometrial concentrations of ormetoprim-sulfadimethoxine in mares. *Can J Vet Res* 53:12.
- Ensink JM, et al. 2003. Clinical efficacy of trimethoprim-sulfadiazine and procaine penicillin G in a *Streptococcus equi* subsp. *zooepidemicus* infection model in ponies. *J Vet Pharm Therap* 26:247.
- Ensink JM, et al. 2005. Clinical efficacy of prophylactic

- administration of trimethoprim/sulfadiazine in a *Streptococcus equi* subsp. *zooepidemicus* infection model in ponies. *J Vet Pharm Therap* 28:45.
- Greko C, et al. 2002. Efficacy of trimethoprim-sulfadoxine against *Escherichia coli* in a tissue cage model in calves. *J Vet Pharm Therap* 25:413.
- Hughes WT. 1988. Comparison of dosages, intervals, and drugs in the prevention of *Pneumocystis carinii* pneumonia. *Antimicrob Agents Chemother* 32:623.
- Messinger LM, Beale KM. 1993. A blinded comparison of the efficacy of daily and twice daily trimethoprim-sulfadiazine and daily sulfadimethoxine-ormetoprim therapy in the treatment of canine pyoderma. *Vet Dermatol* 4:13.
- Peyrou M, et al. 2003. Évolution de la résistance bactérienne envers certains agents antibactériens chez les chevaux dans un center hospitalier vétérinaire. *Can Vet J* 44:978.
- Pohlenz-Zertuche HO, et al. 1992. Serum and skin concentrations after multiple-dose oral administration of trimethoprim-sulfadiazine in dogs. *Am J Vet Res* 53:1273.
- Shpigel NY, et al. 1998. Relationship between in vitro sensitivity of coliform pathogens in the udder and the outcome of treatment for clinical mastitis. *Vet Rec* 142:135.
- Speakman AJ, et al. Antibiotic susceptibility of canine *Bordetella bronchiseptica* isolates. *Vet Microbiol* 71:193.
- Van Duijkeren E, et al. 1994. A comparative study of the pharmacokinetics of intravenous and oral trimethoprim/sulfadiazine formulations in the horse. *J Vet Pharm Ther* 17:440.
- Van Duijkeren E, et al. 1995. Pharmacokinetics and therapeutic potential for repeated oral doses of trimethoprim/sulphachlorpyridazine in horses. *Vet Rec* 137:483.
- White DG, et al. 1998. Comparison of danofloxacin with baquilogrim/sulphadimidine for the treatment of experimentally induced *Escherichia coli* diarrhoea in calves. *Vet Rec* 143:273.
- Williams RB. 2005. The efficacy of a mixture of trimethoprim and sulphaquinoxaline against *Plasmodium gallinaceum* malaria in the domesticated fowl *Gallus gallus*. *Vet Parasitol* 129:193.
- Wilson WD, et al. 1996. Case control and historical cohort study of diarrhea associated with administration of trimethoprim-potentiated sulfonamides to horses and ponies. *J Vet Intern Med* 10:258.

Antiprotozoal Diaminopyrimidines

Some diaminopyrimidines such as pyrimethamine have high activity against protozoa by inhibiting dihydrofolate reductase and thus preventing purine synthesis. These drugs are used in the treatment of systemic protozoal infections such as toxoplasmosis, neosporosis, and equine protozoal myelitis.

Pyrimethamine and sulfadiazine are the most effective drugs in the treatment of toxoplasmosis in humans

and are generally preferred over alternatives such as azithromycin and trimethoprim-sulfamethoxazole. The adult human dosage is 75 mg pyrimethamine and 4 g sulfadiazine PO per day in four divided doses, administered for up to 4 weeks. Dapsone combined with pyrimethamine has good activity experimentally against *Toxoplasma* (Derouin et al., 1991).

Pyrimethamine combined with trimethoprim-sulfadiazine or with an oral sulfonamide alone (20 mg/kg q 24 hours) has become the standard treatment for equine protozoal myeloencephalitis (EPM). Current maintenance dosage is 1 mg/kg daily given orally with trimethoprim-sulfadiazine or -sulfamethoxazole (20 mg/kg daily) for a minimum of 4 months (Fenger, 1997). The trimethoprim component is unnecessary. Anti-inflammatory drugs may also be administered. A small proportion of horses may develop anemia during treatment. Such animals can be treated with folic acid (40 mg daily). Alternate drugs for the treatment of EPM are required, since pyrimethamine is teratogenic for animals and may lead to myeloid, erythroid or lymphoid hypoplasia with epithelial dysplasia and renal hypoplasia or nephrosis in newborn foals. Such effects may be exacerbated by administering folic acid to mares being treated for EPM (Toribio et al., 1998).

Pyrimethamine and diaveridine are commonly combined with sulfaquinoxaline for their synergistic effect against coccidia. Pyrimethamine (1 mg/kg daily) combined with a sulfadoxine (20 mg/kg daily) or trimethoprim-sulfadiazine has been used successfully in the treatment of *Neospora caninum* infection in dogs (Thate and Laanen, 1998).

Bibliography

- Boy MG, et al. 1990. Protozoal encephalomyelitis in horses: 82 cases (1976-1986). *J Am Vet Med Assoc* 196:632.
- Clarke CR, et al. 1992. Pharmacokinetics, penetration into cerebrospinal fluid, and hematologic effects after multiple oral administrations of pyrimethamine to horses. *Am J Vet Res* 53:2296.
- Derouin F, et al. 1991. Anti-*Toxoplasma* effects of dapsone alone and combined with pyrimethamine. *Antimicrob Agents Chemother* 35:252.
- Fenger CK. 1998. Treatment of equine protozoal myeloencephalitis. *Comp Cont Ed Pract Vet* 21:1154.
- Thate FM, Laanen SC. 1998. Successful treatment of neosporosis in an adult dog. *Vet Quart* 20:S113.
- Toribio RE, et al. 1998. Congenital defects in newborn foals of mares treated for equine protozoal myeloencephalitis during pregnancy. *J Am Vet Med Assoc* 212:697.

Fluoroquinolones

Robert D. Walker and Patricia M. Dowling

The fluoroquinolones, also known as quinolones, 4-quinolones, pyridine- β -carboxylic acids and quinolone carboxylic acids are a large and expanding group of synthetic antimicrobial agents. The first of these compounds, nalidixic acid, was first described in 1962, introduced into clinical practice in 1963 and approved for clinical use in 1965. Nalidixic acid had limited clinical application due to its poor absorption following oral administration, moderate antibacterial activity (MICs of 4–16 $\mu\text{g/ml}$ for Enterobacteriaceae), high protein binding (92–97%), and poor tolerance (Bryskier, 2005). Attempts to develop an intravenous form of nalidixic acid administration were unsuccessful, primarily because of limited antibacterial activity and high protein binding.

Between the mid 1960s and the early 1980s there were several other quinolones approved for clinical use, e.g., oxolinic acid, pipemidic acid, piromidic acid, and flumequine. These drugs exhibited increased antibacterial activity but still had limited absorption and distribution. In the 1980s, the addition of both a fluorine molecule at the 6-position of the basic quinolone structure and a piperazine substitution at the 7-position enhanced the antibacterial activity of these compounds, including activity against organisms such as *Pseudomonas aeruginosa* and staphylococci. These modifications also increased the oral absorption and tissue distribution (Ball, 2000). The quinolone nucleus possessing the fluorine molecule gave the group the name “fluoroquinolones”. The first fluoroquinolone approved for use in clinical medicine was norfloxacin, followed shortly thereafter by ciprofloxacin. The first fluoroquinolone approved for use in animals was enrofloxacin, which was approved for use in the United States in companion animals in 1988. Since the approval of enrofloxacin, seven other fluoroquinolones

have been approved for use in companion and food animals.

The fluoroquinolones that are marketed for use in veterinary medicine today are typically well absorbed orally, have a large volume of distribution, penetrate nearly every tissue and cell in the body, and have extended elimination half-lives, allowing for every 24 or 48 hour dosing. At appropriate drug concentration: MIC ratios, the fluoroquinolones are rapidly bactericidal, exhibit concentration-dependent killing, and may exhibit a prolonged in vivo post-antibiotic effect (PAE) on certain bacteria. However, the potential for fairly rapid selection of resistance in some pathogens is a disadvantage of this class of drugs. This can be minimized by appropriate dose selection directed against the right pathogen for the right infectious disease process.

The fluoroquinolones are classified into different groups based on their chemical structure or their biological activities. Classification by chemical structure is dependent on the number of rings associated with the pyridine- β -carboxylic acid nucleus (Bryskier, 2005). Group I is composed of monocyclic derivatives. Group II, which is the majority of fluoroquinolones on the market today, is composed of bicyclic derivatives. This group is divided into two subgroups based on substitutions at position 8 of the quinolone nucleus. Group III is composed of tricyclic derivatives and includes marbofloxacin. Group IV is comprised of those molecules that are quadricyclic, of which only a few have been synthesized and none are marketed for use in veterinary medicine.

The biological classification places the 4-quinolones in three groups. Group I is composed of quinolones with antibacterial activity restricted to the Enterobacteriaceae (e.g., nalidixic acid and flumequine), and

may be divided into those molecules that are metabolized and those that are not (e.g., oxolinic acid and pipemidic acid), respectively. Group II is comprised of those molecules with an extended spectrum of antibacterial activity. The majority of fluoroquinolones are in this group, including all but one of the fluoroquinolones approved for use in veterinary medicine. This group may also be subdivided into Groups IIA and IIB, depending on whether or not they are metabolized. Group III is comprised of those molecules whose antibacterial spectrum also includes organisms such as the streptococci and strict anaerobes. This group is also divided into subgroups IIIA and IIIB according to the metabolism of the molecule. Pradofloxacin is the only Group III fluoroquinolone approved for use in animals. The fluoroquinolones can also be grouped according to their physiochemical properties (Bryskier, 2005). Newer compounds are being explored that optimize the various substitutions and allow for the fluorine atom at position 6 to be replaced, which may reduce side effects, decrease metabolism and decrease interactions with other drugs. The emergence of resistant bacterial strains, however, remains problematic.

To date there have been eight fluoroquinolones approved for use in veterinary medicine: danofloxacin, difloxacin, enrofloxacin, ibafloxacin (Europe only at this time), marbofloxacin, orbifloxacin, pradofloxacin (Europe only at this time), and sarafloxacin. These fluoroquinolones and their current clinical uses in veterinary medicine are listed in Table 17.1. Of these products, sarafloxacin has been voluntarily withdrawn from the market in the U.S. following a request by the Food and Drug Administration's Center for Veterinary Medicine. The use of enrofloxacin in poultry in the U.S. has been withdrawn following a Judicial Review (Federal Register, 2000). This chapter reviews chemical, microbiological, pharmacokinetic, pharmacodynamic, and clinical aspects of the fluoroquinolone antibacterial agents, with specific attention to those agents approved for use in animals (Table 17.1).

Chemistry

The fluoroquinolones, like sulfonamide and nitrofurans, are synthetic compounds (Grohe, 1998). The first clinically approved 4-quinolone-type compound was nalidixic acid. Nalidixic acid lacked several of the char-

Table 17.1. Fluoroquinolones used in veterinary medicine.

Fluoroquinolone	Comments
Enrofloxacin	Approved for use in dogs and cats. In the United States and Canada only approved for respiratory disease in cattle and cannot be used extra-label in food animals in the United States. Approved uses vary widely between other countries with some approvals for lactating dairy cows, swine and poultry. Used extra-label in horses and exotic animals.
Ciprofloxacin	Only approved for humans, but used extra-label in small animals.
Danofloxacin	Cannot be used extra-label in food animals in the United States. Only approved for respiratory disease in cattle in the United States and Canada, but approved for use in cattle, swine and poultry in Europe.
Difloxacin	Only available as small animal oral formulations in the United States and Canada, but cattle and dog injectable formulations are available in Europe. Used extra-label in horses.
Ibafloxacin	Oral formulation available for small animals in Europe.
Marbofloxacin	Only available as small animal oral formulations in the United States and Canada, but large animal injectable formulations are available in Europe. Used extra-label in horses.
Pradofloxacin	Oral formulations for use in dogs and cats in Europe.
Orbifloxacin	Only available as small animal oral formulations. Used extra-label in horses.

acteristics associated with the fluoroquinolones. For example, nalidixic acid has a nitrogen atom at position 8 instead of a carbon atom. With a nitrogen atom at position 1, nalidixic acid has two nitrogen atoms in its basic nucleus, making it a naphthyridone molecule rather than a quinolone molecule. In addition, nalidixic acid is not halogenated like other quinolones. Since the discovery of nalidixic acid's antibacterial activities, more than 10,000 compounds have been designed from the parent bicyclic 4-quinolone molecule. Today the majority of fluoroquinolones marketed for clinical use in veterinary medicine are bicyclic derivatives. One exception is marbofloxacin, which is a tricyclic molecule (Figure 17.1).

Clinically, nalidixic acid has several limitations. These include a narrow spectrum of activity, poor pharmacokinetic properties, toxic effects, and a tendency to select for resistant organisms. Replacing the hydrogen atom at position 6 of the 4-quinolone molecule with a fluorine atom resulted in increased activity against both Gram-positive and Gram-negative bacteria. The increased activity is attributed to in-

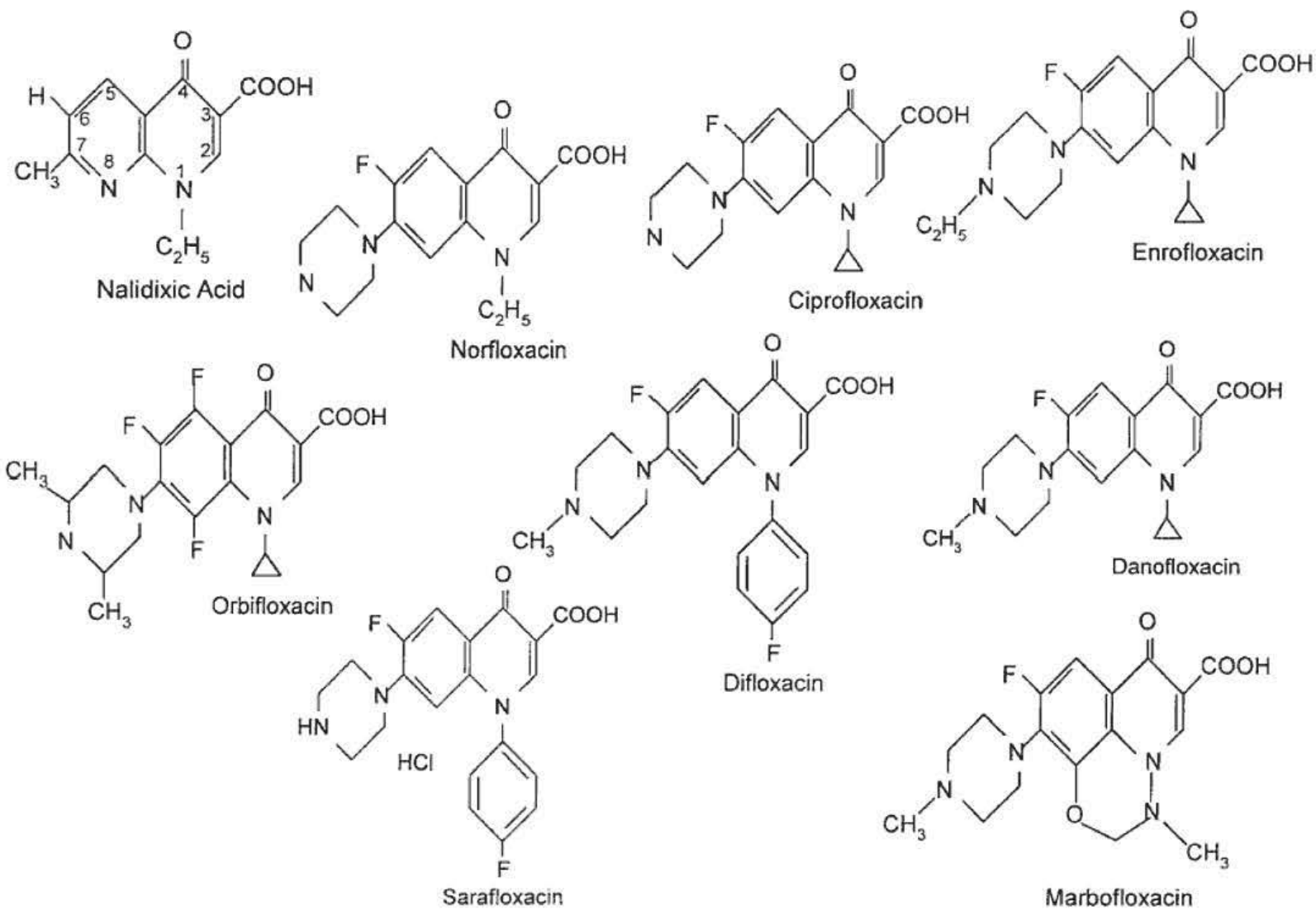


Figure 17.1. Structures of fluoroquinolones used in veterinary medicine.

creased penetration of the bacterial cell membrane (Petersen and Schenke, 1998).

Substituting a piperazinyl ring for the methyl group at position 7 increased Gram-negative activity, including anti-pseudomonal activity. These modifications led to the development of the first broad-spectrum fluoroquinolone, norfloxacin, which was marketed in 1986. Additional studies demonstrated that substantial changes in potency could be obtained by variations at the N-1 and C-7 positions. For example, ciprofloxacin is similar in structure to norfloxacin but has a cyclopropyl group in place of the ethyl group at N-1. This substitution enhances ciprofloxacin's Gram-positive and Gram-negative activity. This cyclopropyl group is also found on enrofloxacin, danofloxacin, pradofloxacin and orbifloxacin. Difloxacin has a phenyl ring at position N-1 that reportedly gives it enhanced activity against Gram-positive bacteria, relative to enrofloxacin activity. Difloxacin also has a second fluorine atom in its structure, whereas orbifloxacin has a total of three fluorine atoms. These additional fluorine atoms do not appear to influence the antibacterial activity of these compounds.

Overall, there have been several chemical modifications at each of the eight positions in the 4-quinolone molecule. Some increase absorption, some increase antibacterial activity, and others increase toxicity. For example, ciprofloxacin and enrofloxacin are similar molecules except for the ethyl group on the piperazinyl ring of enrofloxacin. This ethyl group enhances the oral absorption of enrofloxacin over ciprofloxacin in the dog but decreases its anti-pseudomonal activity (Walker et al., 1990; 1992).

Mechanism of Action

The bacterial chromosome is a continuous, circular, double-stranded DNA molecule approximately 1,000 times longer than the bacteria in which it is contained. In order for such a long molecule to fit into the cell, it is densely packed in a negative supercoil, twisted in the opposite direction to the right-handed double helix of DNA. This supercoiled configuration is so highly strained that to improve function, the chromosome is divided into approximately 50 topologically independent domains. Topoisomerase enzymes catalyze changes in coiling of the molecule. Topoisomerase I is characterized by reactions involving single-stranded DNA,

whereas topoisomerase II is involved in reactions with double-stranded DNA. Topoisomerase II, also known as DNA gyrase, consists of two subunits, GyrA and GyrB. The *gyrA* gene encodes two α -subunits while the *gyrB* gene encodes two β -subunits; the active DNA gyrase is an A_2B_2 complex. DNA gyrase binds to DNA; a segment of approximately 130 nucleotides wraps around the DNA gyrase. This wrapped DNA is cleaved in both strands, forming a DNA-protein covalent bond between the GyrA subunit and the 5'-phosphates of the DNA molecule. Another segment of DNA is passed through this double-stranded break, which may then be resealed. The α -subunit of the DNA gyrase is important in the breakage and reunion that allow for this relaxation of the DNA molecule. In multiple species of bacteria, it has been shown that the 4-quinolone molecule interrupts the DNA breakage-reunion step by binding to the DNA gyrase-DNA complex, and thus leading to defects in negative supercoiling.

Studies have also shown that the fluoroquinolones may have a second intracellular target, DNA topoisomerase IV (Topo IV) (Kato et al., 1990, 1992). This is a bacterial type II DNA topoisomerase and is also a multimeric protein composed of two ParC subunits and two ParE subunits, which exhibit sequence homology to GyrA and GyrB, respectively. This enzyme mediates relaxation of duplex DNA and the unlinking of daughter chromosomes following replication (Zechiedrich and Cozzarelli, 1995). However, unlike the DNA gyrase, Topo IV cannot supercoil DNA. Instead it is involved in the ATP-dependent relaxation of DNA. It is a more potent decatenase than DNA gyrase (Hoshino et al., 1994). Topo IV may be the primary target of fluoroquinolones in *S. aureus* and streptococci (Ferrero et al., 1994; Kaatz and Seo, 1998). This indicates that the primary target of fluoroquinolones varies in different bacteria.

The effect of fluoroquinolones on bacterial proliferation suggests three mechanisms of cell killing (Martinez et al., 2005; Guthrie et al., 2004; Maxwell and Critchlow, 1998):

1. Mechanism A: common to all quinolones. This requires RNA and protein synthesis and is only effective against dividing bacteria. Mechanism A appears to involve the blocking of replication by the gyrase-quinolone complex on DNA.
2. Mechanism B: does not require RNA and protein synthesis and can act on bacteria that are unable to

multiply. Mechanism B (chloramphenicol insensitive) can be correlated with dislocation of the gyrase sub-units that constrain the ternary complex.

3. Mechanism C: requires RNA and protein synthesis, but does not require cell division. Mechanism C may correlate with trapping of topo IV complexes on DNA.

Antimicrobial Activity

The fluoroquinolones have excellent activity in vitro against a wide range of aerobic Gram-negative bacteria, including the Enterobacteriaceae, *Actinobacillus pleuropneumoniae*, *Histophilus somni* (*Haemophilus somnus*), *Mannheimia* (*Pasteurella*) *haemolytica*, and *Pasteurella* spp. including *P. multocida*. They are also active against *Bordetella bronchiseptica*, *Brucella* spp., *Chlamydia*/*Chlamydophila* spp., *Mycoplasma* spp., and *Ureaplasma*. Activity against *Pseudomonas aeruginosa* is variable, with ciprofloxacin being the most potent agent against this bacterium (Van Bambeke et al., 2005). For the most part, the older fluoroquinolones are less active against Gram-positive bacteria, especially enterococci, and have poor activity against anaerobic bacteria. Newer fluoroquinolones target this deficiency. For example, trovafloxacin, moxifloxacin, clinafloxacin and sitafloxacin are newer fluoroquinolones with good in vitro activity against strict anaerobes. All of the fluoroquinolones approved for use in veterinary medicine should be considered ineffective against the strict anaerobes (Bryskier, 2005; Goldstein et al., 1998). The possible exception may be pradofloxacin (based on its structure, which is very similar to moxifloxacin, although specific data to support this is not available at this time).

The in vitro activities of fluoroquinolones used in veterinary medicine are listed in Table 17.2, 17.3 and 17.4. Because the susceptibility of some bacterial isolates of animal origin to the fluoroquinolones decreases over time (see Table 2.2; Walker and Thornberry, 1998), the values listed in the table need to be evaluated in relation to the isolation date of the organisms.

Fluoroquinolones exhibit a biphasic dose/response curve (paradoxical effect) in that they are less active at concentrations below, equal to or much higher than the MIC (Martinez et al., 2005; Brown, 1996). As the ratio of fluoroquinolone concentration to MIC in-

creases from $\leq 1:1$ to the optimal bactericidal concentration (usually approximately 10:1 to 12:1, though this may be drug- and bacterium-dependent), bacterial killing increases and is usually very rapid (Maxwell and Critchlow, 1998; Preston et al., 1998).

As illustrated in Figure 17.2, when a strain of *M. haemolytica* is exposed to a fluoroquinolone at concentrations that are 25% of its MIC, the drug exhibits a slight stationary effect, but then the bacterium resumes growth at a rate similar to that of the untreated control. As the concentration of the drug is increased above the MIC, there is a decrease in the number of viable organisms. For drug concentrations that are equivalent to the MIC, there is a slight decrease in the number of viable organisms, but after 24 hours of exposure the number of viable organisms increases to more than what was in the starting suspension. This occurs without an increase in MICs. This suggests that fluoroquinolones, at concentrations that are equal to the MIC, have a static effect on *M. haemolytica*. When the concentration of the fluoroquinolone is increased to four times the MIC, there is a nearly 4 \log_{10} reduction in the number of viable organisms within four hours of exposure. However, this killing effect stabilizes and then the organisms begin to proliferate, again without an increase in MIC. This is in contrast to the growth rate when the concentration of the fluoroquinolone is eight times the MIC. Under this circumstance, there is a very rapid bactericidal effect and 7-log reduction in viable organisms, and after a 24-hour exposure there is no detectable bacterial re-growth. This suggests that at eight times the MIC, there is a 100% bactericidal effect. The concentration-dependent killing effect may plateau at fluoroquinolone concentrations 15 to 20 times MIC, above which the fluoroquinolones may become bacteriostatic (Schentag and Scully, 1999). Others, however, have not observed this paradoxical (Eagle) effect, even at concentrations 200 times the MIC (Gould et al., 1990).

The decrease in antibacterial activity at high drug concentrations is thought to be caused by the inhibition of RNA and protein. This implies that protein synthesis may be required for quinolone-mediated cell death. In this regard, it has been reported that protein synthesis inhibitors (such as chloramphenicol) and RNA synthesis inhibitors (such as rifampin) may reduce fluoroquinolone effectiveness in bacterial killing, but this has not been demonstrated clinically (Guthrie et al., 2004; Maxwell and Critchlow, 1998).

Table 17.2. Microbiological activity (MIC₉₀ µg/ml) of the fluoroquinolones to common bacterial pathogens isolated from animals.

Organism	Enrofloxacin ^a		Orbifloxacin ^b		Ibafloxacin ^c		Difloxacin ^d		Ciprofloxacin ^e		Pradofloxacin ^f	
	MIC ₉₀ Range	No. Isolates	MIC ₉₀ Range	No. Isolates	MIC ₉₀ Range	No. Isolates	MIC ₉₀ Range	No. Isolates	MIC ₉₀ Range	No. Isolates	MIC ₉₀ Range	No. Isolates
<i>Bordetella bronchiseptica</i>	0.5-2.0	273			8	35	2-8	53	1	43	0.25	144
<i>Staphylococcus intermedius</i>	0.12-0.5	349	0.5-1.0	321	0.25	281	0.125-1.0	186	0.25	25	0.06	1606
<i>Staphylococcus aureus</i>	0.12-0.25	202	0.5	15	0.25	86			0.5	50	0.5	269
<i>Streptococcus canis</i>			1.0-2.0	36	32	34	2	17				
<i>Enterococcus</i> spp.	1-2	59	16-32	35	4	31						
<i>Escherichia coli</i>	0.03-0.125	529	0.5	78	0.5	150	0.125-0.25	81	≤0.015-0.06	95	2	1239
<i>Klebsiella pneumoniae</i>	0.06-0.12	104	0.25	12	0.5	24	0.5	20	0.06	37	0.25	38
<i>Proteus</i> spp.	0.12-0.5	147	1.0-2.0	24	0.5	43	1-4	48	0.03-0.06	58	4	121
<i>Pseudomonas</i> spp.	1-8	246	8-16	17	16	45	4	24	0.12	50	2	451

^aCarbone et al., 2001; Lautzenhiser et al., 2001; Speakman et al., 1997; Speakman et al., 2000; Walker 1998-99 unpublished data; Watts, 1997.

^bGaniere et al., 2004; Technical monograph, values adjusted to CLSI dilution schemes.

^cCoulet et al., 2002.

^dCarbone et al., 2001; van den Hoven et al., 2000.

^eCarbone et al., 2001; Walker et al., 1990; Watts et al., 1997; adjusted to CLSI dilution values.

^fdeJong, 2004.

*MIC₉₀ is presented for all organisms where more than 15 isolates were tested. MIC₉₀ range is presented if the data were generated from more than one study and each study presented a different MIC₉₀.

Table 17.3. Susceptibility of bovine bacterial pathogens to marbofloxacin.^a

Organism	Year Isolated	N	MIC µg/ml		
			≤ 0.06	0.12 – 1	≤ 2
<i>Escherichia coli</i> (enteric)	2000	151	93 ^b (62) ^c	35 (85)	23 (100)
	2001	79	46 (58)	19 (82)	14 (100)
<i>E. coli</i> (mastitis)	2000	102	100 (98)	2 (100)	
	2001	96	93 (97)	2 (99)	1 (100)
<i>Salmonella</i> spp.	2000	57	50 (88)	7 (100)	
	2001	49	43 (88)	6 (100)	
<i>Mannheimia haemolytica</i>	2000	81	52 (64)	24 (94)	5 (100)
	2001	30	12 (40)	15 (90)	3 (100)
<i>Pasteurella multocida</i>	2000	109	94 (86)	14 (99)	1 (100)
	2001	67	56 (84)	11 (100)	
<i>Staphylococcus aureus</i>	2000	67	2 (3)	65 (100)	
	2001	45		45 (100)	
<i>Streptococcus</i> spp. ^b	2000	102	100 (98)	2 (100)	
	2001	96	93 (97)	2 (99)	1 (100)

^aFrom Meunier et al., 2004.^bNumber of isolates.^cCumulative percentage.^d*Streptococcus* isolates tested include *S. agalactiae*, *S. dysgalactiae* and *S. uberis*.**Table 17.4.** Susceptibility of various canine and feline bacterial pathogens to marbofloxacin.^a

Organism	Year Isolated	N	MIC µg/ml		
			≤ 0.06	0.12 – 1	≤ 2
<i>Escherichia coli</i>	1999	22	18 ^b (82) ^c	1 (86)	3 (100)
	2000	34	27 (79)	3 (88)	4 (100)
	2001	20	17 (85)	1 (90)	2 (100)
<i>Pseudomonas aeruginosa</i> , skin	1999	33		30 (91)	3 (100)
	2000				
	2001	29 ^d		27 (93)	2 (100)
<i>P. aeruginosa</i> , otitis	1999	21		18 (86)	3 (100)
	2000	16		16 (100)	
	2001	23		17 (74)	6 (100)
<i>Staphylococcus intermedius</i>	1999	33		32 (97)	1 (100)
	2000	33		33 (100)	
	2001	19		19 (100)	

^aMeunier et al., 2004.^bNumber of isolates.^cCumulative percentage.^dRepresents isolates from 2000 and 2001.

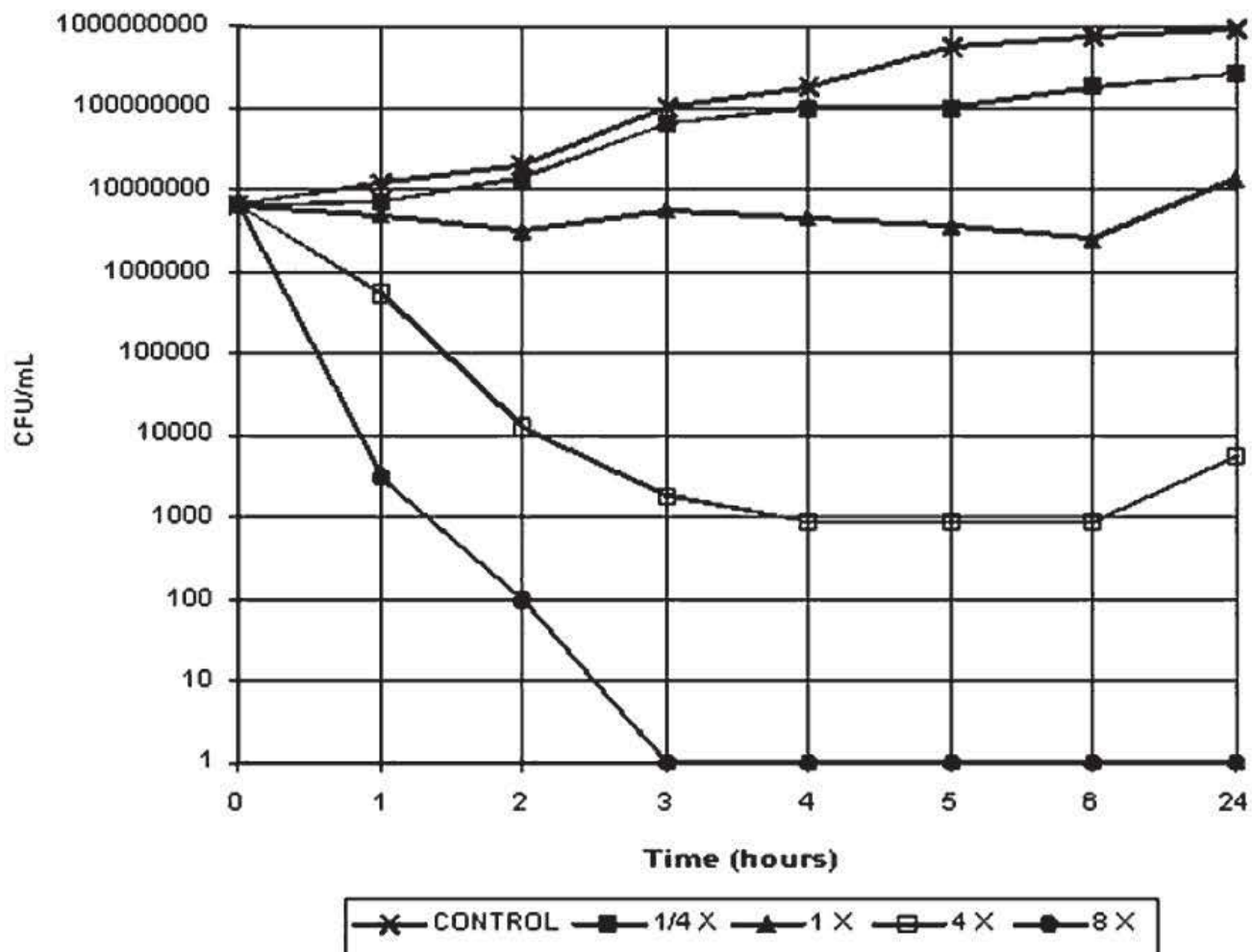


Figure 17.2. Concentration-dependent killing effect of a fluoroquinolone tested against *Mannheimia (Pasteurella) haemolytica*.

While the antibacterial activity of the fluoroquinolones is dependent on the drug concentration relative to the MIC of the bacterium, the MIC is independent of the bacterial concentrations. As the bacterial concentration increases from 10^3 to 10^8 colony-forming units/ml (CFU/ml), the MIC remains constant. This is not the case with the minimal bactericidal concentration (MBC). As the bacterial concentration increases from 10^8 to 10^{10} CFU/ml, fluoroquinolone activity goes from decreased bactericidal activity to bacteriostatic (Bryskier, 2005). This phenomenon may be related to oxygen depletion by bacterial metabolism, as under anaerobic conditions ciprofloxacin becomes bacteriostatic.

Bacterial Resistance

Resistance to fluoroquinolones occurs by target modification, decreased permeability, efflux pumps, and/or target protection. Each of these fluoroquinolone resistance mechanisms can occur simultaneously within the same cell, thereby leading to very high resistance levels. To date, no mechanisms based on enzymatic inactivation/modification of fluoroquinolones have been discovered. Because fluoroquinolones are synthetic antimicrobials with no known natural analogues, it is less likely that this type of mechanism will emerge. Selection of resistant mutants by decreased permeability or efflux mechanisms generally means a 2- to 8-fold increase in MIC, whereas alteration of the

DNA gyrase binding site or target protection may result in high-level resistance.

Resistance to one fluoroquinolone frequently results in resistance to all. This is especially true for the older compounds and for high-level resistance. Fluoroquinolone resistance due to target mutations typically results in decreased susceptibility or resistance to other fluoroquinolones. Resistance due to alterations in permeability or activation of an efflux pump can confer resistance to other antimicrobial agents, such as the cephalosporins, carbapenems, and tetracyclines (Everett et al., 1996; Piddock et al., 1998; Poole, 2000; Van Bambeke et al., 2005).

Because fluoroquinolones mediate DNA damage by binding to susceptible enzymes, fluoroquinolone-resistance mutations are recessive. For topoisomerase-mediated fluoroquinolone resistance to be transferred horizontally, an acquired mutated gene must supplant the wild-type gene.

The development of fluoroquinolone resistance via mutations in topoisomerases has been studied extensively. Resistance is mediated primarily by target mutations in DNA gyrase (topoisomerase II) (Nakamura et al., 1989; Yoshida et al., 1990), with secondary mutations in topoisomerase IV contributing to higher levels of resistance (Vila et al., 1996). Amino acid substitutions that result in bacterial resistance have been localized to a specific topoisomerase subdomain termed the quinolone resistance-determining region (QRDR), located within *gyrA* (Yoshida et al., 1988; 1990) and *parC* (Khodursky et al., 1995). In *E. coli*, most mutations associated with quinolone resistance occur in the QRDR at serine 83 (Ser83) and aspartate 87 of *gyrA*, and at serine 79 and aspartate 83 of *parC*, as well as at analogous sites in other species (Bebear et al., 2003; Takiff et al., 1994; Taylor and Chau, 1997). DNA sequence analysis of *S. aureus* and *Streptococcus* genes shows that the situation can be reversed in Gram-positive bacteria, where topoisomerase IV (encoded by *griA* and *griB*) is the primary fluoroquinolone target (Munoz and De La Campa, 1996; Ng et al., 1996). In both cases, mutations decrease the quinolone affinity for the enzyme/DNA complex (Maxwell and Critchlow, 1998), and allow DNA replication to continue in the presence of fluoroquinolone concentrations that are inhibitory to wild-type cell growth.

In Gram-negative organisms quinolone resistance typically develops in a stepwise manner. A single QRDR mutation, usually at Ser83, confers resistance to nalidixic acid and decreases susceptibility to fluoro-

quinolones (ciprofloxacin MICs may go from a wild-type baseline of 0.015–0.03 µg/ml to 0.125–1.0 µg/ml). Secondary mutations in the *gyrA* QRDR lead to overt fluoroquinolone resistance (ciprofloxacin MICs \geq 4 µg/ml). However, this does not hold true for all Gram-negative bacteria. In *Campylobacter* spp., which lack topoisomerase IV, a single mutation in *gyrA* is sufficient to impart high-level ciprofloxacin MICs (32 µg/ml) (Wang et al., 1993). This feature helps explain the higher prevalence of resistance in *Campylobacter*, compared to *E. coli*, from food animals exposed to fluoroquinolones (Van Boven et al., 2003).

As indicated above, fluoroquinolone resistance may also be mediated by decreased permeability of the bacterial cell wall through altered outer membrane porins (OmpF) and by the activity of energy-dependent efflux pumps. Most fluoroquinolones cross the Gram-negative outer membrane through protein channels called porins (Nikaido and Vaara, 1985), although some may diffuse directly across the lipid bilayer. Resistance due to decreased quinolone influx is generally reflected in low-level changes in susceptibility, and may explain differences in potency among different fluoroquinolone derivatives. Porin deficiency has been associated with quinolone resistance in *E. coli* and *Pseudomonas*. For example, mutations of the *E. coli* porin OmpF produced about a 2-fold increase in quinolone MICs (Aleksun and Levy, 1999).

However, it is difficult to experimentally assess the role of porins without also accounting for effects due to efflux. Permeability changes mediated by altered porins are often part of a coordinated cellular response to the presence of numerous toxic agents, which includes simultaneous up regulation of efflux. In *E. coli*, de-repression in regulatory loci such as *marA* or *soxS* leads to decreased fluoroquinolone susceptibility via simultaneous up-regulation of the AcrAB-TolC efflux pump (Okusu et al., 1996) and down-regulation of the OmpF porin (Cohen et al., 1988). This mechanism confers decreased susceptibility to a large number of other antimicrobial agents in addition to fluoroquinolones. Analogous regulatory loci exist among other species of bacteria (Cohen et al., 1993).

In antimicrobial efflux systems, membrane-localized proteins actively pump drug from the cell before it can diffuse to its primary target within the active site of DNA gyrase. Because they are driven by the proton motive force, energy uncouplers can be used to study their role in resistance. The *E. coli* genome carries as

many as 30 potential efflux pumps, many of which mediate antimicrobial efflux. Some are effective for specific agents, whereas others protect against a variety of structurally diverse compounds. In addition, a single bacterium may contain multiple efflux pumps (e.g., AcrAB and CmlA) that are capable of extruding the same antimicrobial agent.

Constitutive and inducible efflux is a known mechanism of fluoroquinolone resistance in both Gram-negative and Gram-positive bacteria, and may be more important than secondary mutations in topoisomerase IV genes. For example, it has been shown that deletion of the gene encoding the inducible AcrAB efflux pump reduces ciprofloxacin MICs to near wild-type levels in cells carrying topoisomerase mutations (Oethinger et al., 2000). In *Campylobacter*, where efflux mediated by CmeAB is constitutive, fluoroquinolone MICs in wild-type cells are 3- to 4-fold higher than those typical of *E. coli*. Insertional inactivation of CmeAB in *C. jejuni* reduces ciprofloxacin MICs to levels near that of wild-type *E. coli* (0.003 µg/ml) (Luo et al., 2003). These findings have led some drug developers to examine bacterial efflux systems as potential targets for antimicrobials.

Bacterial fluoroquinolone resistance was once thought to disseminate exclusively via clonal expansion under selective pressure. Recently, a plasmid-mediated quinolone resistance gene (*qnr*) was described, first in clinical isolates of *Klebsiella pneumoniae* (Martinez-Martinez et al., 1998) and later in *E. coli* (Jacoby et al., 2003; Wang et al., 2003). The *qnr* gene is located near sequences (*qacEA*⁺ 1, *sulI*) typically associated with class I integrons: the *qnr* gene encodes a 218-amino-acid protein belonging to the pentapeptide repeat family (Tran and Jacoby, 2002). In a concentration-dependent manner, *qnr* functions by protecting *E. coli* DNA gyrase, but not topoisomerase IV, from inhibition by ciprofloxacin (Tran and Jacoby, 2002). The *qnr* gene confers a small decrease in quinolone susceptibility, such that *qnr*⁺ strains are still considered clinically susceptible. The presence of *qnr* permits selection of topoisomerase mutants at concentrations that normally would be toxic to the bacterium (Martinez-Martinez et al., 1998).

Pharmacokinetic Properties

The fluorinolones are absorbed rapidly and well from the gastrointestinal tract of monogastric animals and

pre-ruminant calves. Enrofloxacin is more lipid soluble than ciprofloxacin and has a higher oral bioavailability than ciprofloxacin in horses and small animals. All of the oral veterinary products typically have high bioavailability in dogs and cats, but enrofloxacin bioavailability was poor in neonatal kittens (Seguin et al., 2004). The oral bioavailability of enrofloxacin is approximately 60% in adult horses and 42% in foals. While it is extremely low in adult cattle, it is surprisingly good in sheep (80%). The pharmacokinetic parameters of fluoroquinolones administered to dogs, cattle, horses, and pigs are given in Table 17.5.

Ingestion with food may delay the time to peak serum concentrations without affecting total serum concentrations, unless the food is rich in magnesium or aluminum ions. Increases in oral dose usually produce linear increases in serum concentrations.

Following absorption, fluoroquinolones exhibit rapid and extensive tissue distribution because of their hydrophilic nature and low (<50%) protein binding. Their apparent volumes of distribution exceed total body water (> 1 L/kg). In general, fluoroquinolone concentrations in interstitial fluid, skin, and bones are 35-100% of those obtained in the serum, whereas bronchial secretions and prostatic concentrations may be two to three times the corresponding serum concentrations. Penetration into cerebrospinal fluid is approximately 25% of serum concentration. Therapeutic concentrations for Gram-negative bacteria may be achieved in the CSF and ocular fluids. High concentrations are found in the bile and organs of excretion (liver, intestine, and urinary tract).

The fluoroquinolones are concentrated within phagocytic cells. Uptake occurs by simple diffusion, and intracellular concentrations may be several times greater than plasma concentrations. Intracellular drug is microbiologically active; in vitro studies indicate that ciprofloxacin reduces survival of intracellular pathogens such as *Brucella* spp., *Mycoplasma* spp., and *Mycobacterium* spp.

The fluoroquinolones are largely excreted unchanged in the urine by glomerular filtration and active tubular secretion. The exception is difloxacin, where 80% is excreted in the feces. Metabolites and the parent compound may be excreted in an active form in the bile and urine. For example, the major metabolite of enrofloxacin is ciprofloxacin. The amount of ciprofloxacin produced varies among species, with some producing ciprofloxacin concentrations that ex-

Table 17.5. Comparative pharmacokinetic parameters of selected fluoroquinolones administered orally to cats, dogs, horses, cattle, and pigs.

Fluoroquinolone	Animal Species	Route	Dose* (mg/kg)	C _{MAX} (µg/ml)	Vd (L/kg)	T _{1/2B} (hr)	AUC ₀₋₂₄ (µg·h/ml)	Bioavailability (%)
Ciprofloxacin	Cats	IV	10		3.9	4.5	17	
		PO	10	1.26		3.7	11	33
	Dogs	IV	10	3.1	2.2			
		PO	10	1.55		4.9		
Enrofloxacin	Ponies	IV	5		3.45	2.5		6
	Cats	IV	5		2.37	6.7	18.6	
	Kittens (2 wks old)	IV	5		1.8	4.2	16.7	
		PO	5	0.5		4.8	5.7	33.7
	Dogs	IV	5		3.7	2.4		
		PO	5	1.41		4.1	8.74	83
	Horses	IV	5		2.3	4.4		
		PO	5	5.4		6.1	35.6	63
	Foals	IV	5		2.47	17.1	48.54	
		PO	10	2.12		18.4	58.47	42
	Cattle	IV	5		4.0	2.6	4.4	
		SC	8	0.81		7.3	7.51	
Danofloxacin	Pigs	IV	5		6.11	10.5	11.2	
		PO	10	1.4				83
	Cattle	SC	8	2.4		3.8	14.76	
	Dogs	PO	5	1.1	4.7	6.9	9.34	
Ibafloxacin	Cats	PO	15	6.86			37.14	
	Dogs	IV	15		1.14	5.2	29.13	
		PO	15	6.04		3.4	21.28	69.1
Marbofloxacin	Cats	IV	2		1.01	7.9	21.26	
		PO	2	2.34		7.8	24.73	100
	Dogs	IV	2		1.37	12.4		
		PO	2	1.47		9.1	13.07	94
	Cattle	IM	2	1.98		6.3	7.65	
Orbifloxacin	Cats	IV	2.5		1.3	4.5	10.6	
		PO	2.5	2.06		5.5	10.82	~100
	Dogs	IV	2.5		1.2	5.4	14.3	
		PO	2.5	1.37		7.1	12.72	~100

ceed the MIC of some pathogens (Kung et al., 1993). The elimination half-life of the fluoroquinolones is dependent on the drug and the animal species, and may be dose-dependent. Long elimination half-lives make the fluoroquinolones ideal for every 24 or 48 hour dosing regimens.

Pharmacodynamic Properties

With ideal pharmacokinetic parameters but a potential to select for resistant bacteria, optimal therapeutic dosage regimens for fluoroquinolones require integration of pharmacokinetics and pharmacodynamics.

Pharmacodynamic indices describe the interaction of drug concentration, which is dependent on dose and pharmacokinetic properties, with the bacterial killing ability of the drug. The bacterial killing ability of a fluoroquinolone is related to the MIC, because the MIC and the MBC are usually within one dilution of each other when the fluoroquinolone MIC is ≥ 4 µg/ml. Although low MICs generally indicate greater in vitro potency, the values must be interpreted in relation to achievable serum and tissue concentrations of the drug.

By simultaneously considering the serum concentration or AUC₀₋₂₄ of the fluoroquinolone and the MIC of the pathogen, an index of beneficial effect can

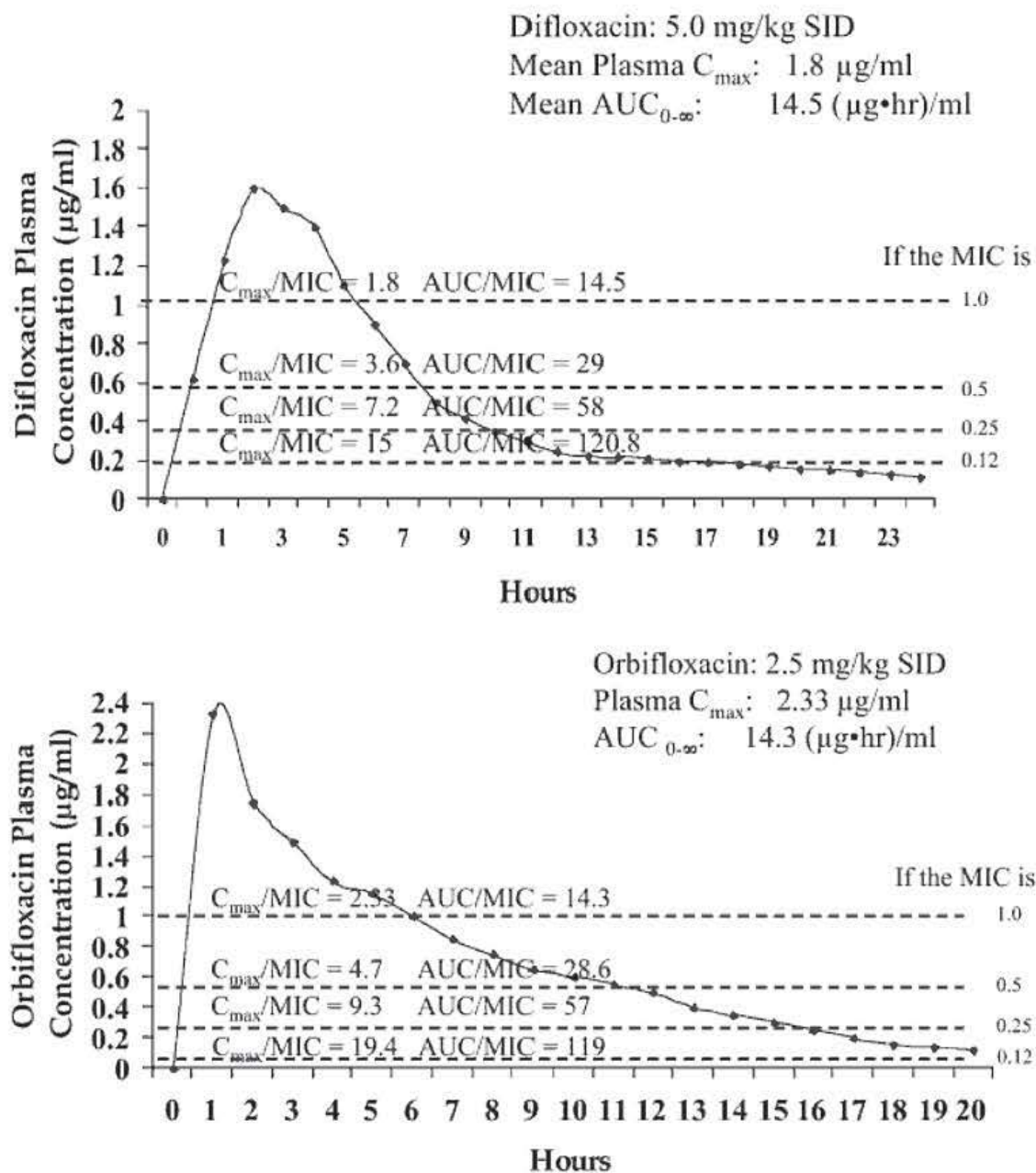


Figure 17.3. Determining peak MIC and AUC_{0-24}/MIC ratios for orbifloxacin. Bioavailability curves, plasma C_{max} , and AUC are from the sponsor's package insert (Orbifloxacin, 1997).

be determined. In the example given in Figure 17.3, peak plasma concentration and $AUC_{0-\infty}$ value are from the low doses of orbifloxacin. (Note that in this bioavailability curve, $AUC_{0-\infty}$ is used instead of AUC_{0-24} , resulting in a slightly higher AUC value and corresponding AUC:MIC value.) The proposed MIC values are in accordance with the Clinical Laboratory Standards Institute (CLSI) dilution schemes and rep-

resent an MIC value received from a package insert or from a microbiology report, provided the laboratory reports MICs. The index of beneficial effect shows that a $AUC_{0-24}/\text{MIC} \geq 125$ (or C_{max}/MIC of 10) is linked with favorable clinical and microbiological outcomes, whereas a $AUC_{0-24}/\text{MIC} < 100$ (or $C_{max}/\text{MIC} < 4$) is associated with sub-optimal clinical and microbiological results (Van Bambeke, 2005). However, these ra-

Table 17.6. Relationship between serum AUC₀₋₂₄ and MIC values.

If the AUC ₀₋₂₄ is...	MIC (μg/ml)							
	0.03	0.06	0.12	0.25	0.5	1.0	2.0	4.0
5	167	83	42	20	10	5	2.5	1.25
10	333	167	83	40	20	10	5	2.5
15	500	250	125	60	30	15	7.5	3.75
20	667	333	167	80	40	20	10	5
25	833	417	208	100	50	25	12.5	6.25
30	1,000	500	250	120	60	30	15	7.5

Note: Values listed are the AUC₀₋₂₄:MIC ratios, at various AUC₀₋₂₄ values with commonly reported MICs that could pertain to any fluoroquinolone at the appropriate dose. These ratios are dependent not on any specific fluoroquinolone but rather on the relationship between the AUC and the MIC.

tios are dependent on the severity of the infection. For example, for less severe infections AUC₀₋₂₄/MIC values of 25 to 50 may be sufficient, whereas AUC₀₋₂₄/MIC values exceeding 125 are required for severe infections or for immunosuppressed patients. Clinical data have shown that for severe infections, when the AUC₀₋₂₄/MIC ratio was ≥ 250 bacterial eradication was faster than when the ratio was 125 (Schentag et al., 2003). Thus, in the example presented here, for those bacterial pathogens with low MICs (0.12 μg/ml or less), the low doses of orbifloxacin should be adequate. Infections caused by pathogens with higher MICs may require higher doses to achieve the optimal clinical outcome. This would be true for all fluoroquinolones.

This point is illustrated by the dose-AUC values listed in Table 17.5 and the AUC:MIC values listed in Table 17.6. As discussed above, AUC₀₋₂₄ depends on dose or frequency of dosing. AUC values increase with increases in dose or with increased dosing frequency. For bacterial pathogens with MICs ≤ 0.06 μg/ml, AUCs of 5 to 10 would result in AUC:MIC ratios of 83 to 333 (Table 17.6). On the other hand, in treating pathogens with higher MICs, the dose or frequency of dosing would need to be increased in order to achieve higher AUC values and thus an effective AUC₀₋₂₄:MIC ratio. For example, if the MIC of a pathogen from a dog was 0.25 μg/ml when tested against enrofloxacin, a 5 mg/kg dose of enrofloxacin may be inadequate, as the AUC:MIC ratio would be less than 40 (Tables 17.5 and 17.6). A dose of 11 mg/kg enrofloxacin would be result in an AUC:MIC ratio of >100 .

The effective use of the fluoroquinolones depends on designing dosing regimens that attain serum AUC₀₋₂₄:MIC ratios ≥ 125 or C_{max}:MIC ratios $\geq 10:1$.

By using the MIC data provided on the package insert, in Tables 17.2–17.4, or in the microbiological report resulting from the submitted sample, along with the pharmacokinetic parameters from the package insert or from Table 17.5, a practitioner should be able to determine the optimal dose to use. Failure to use this approach may result in therapeutic failure and the selection of resistant bacterial pathogens.

Drug Interactions

The fluoroquinolones are synergistic with beta-lactams, aminoglycosides, and vancomycin against some bacterial pathogens. Examples include *Staphylococcus aureus* (ciprofloxacin and azlocillin; levofloxacin and oxacillin), *Pseudomonas aeruginosa* (ciprofloxacin and imipenem, azlocillin or amikacin), and enterococci (ciprofloxacin and ampicillin or vancomycin) (Eliopoulos and Moellering, 1996). Antagonistic interactions have been demonstrated in vitro between ciprofloxacin and chloramphenicol and ciprofloxacin and rifampin (Eliopoulos and Moellering, 1996). Fluoroquinolones have been used with metronidazole to expand the antibacterial spectrum against polymicrobial infections that involve strict anaerobes. Oral administration of the fluoroquinolones with products containing divalent or trivalent cations (such as calcium, iron, magnesium, zinc, or aluminum) may reduce the absorption of the fluoroquinolones. Concurrent administration of fluoroquinolones can reduce elimination of drugs that depend on liver metabolism for excretion. For example, the fluoroquinolones decrease the hepatic clearance and thus increase the

elimination half-life of theophylline and caffeine (Intorre et al., 1995). By inhibiting renal tubular secretion, probenecid has been shown to reduce the renal clearance of ciprofloxacin by 50% in humans (Stein, 1988).

Toxicity and Adverse Effects

Fluoroquinolones are relatively safe antimicrobial drugs. Administered at therapeutic doses, toxic effects are mild and generally limited to gastrointestinal disturbances such as nausea, vomiting, and diarrhea.

Chronic, high-dose fluoroquinolone therapy causes articular cartilage lesions in juvenile dogs, particularly in weight-bearing joints (Burkhardt et al., 1992). No documented arthropathies have been reported for calves, swine, or poultry. Enrofloxacin inhibits cell proliferation, induces morphological changes, decreases total monosaccharide content and alters small proteoglycan synthesis at the glycosylation level in equine tendon cell cultures (Yoon JH et al., 2004). These effects are more pronounced in juvenile tendon cells than in adult equine tendon cells. Arthropathies have been documented in two-week-old foals after receiving 10 mg/kg enrofloxacin orally (Vivrette et al., 2001). Damage was characterized by synovial joint effusion and lameness, erosion, and cleft formation in articular cartilage. Arthropathies were not seen in adult horses that were given up to 25 mg/kg of enrofloxacin IV daily for 3 weeks or 15 mg/kg PO every 12 hours for 3 weeks (Bertone et al., 2000). While not recommended for use in pregnant humans or animals, the fluoroquinolones appear to have little effect on the developing fetus. Enrofloxacin was successfully used to treat chronic pleuritis in a pregnant mare with no apparent detrimental effects on the foal (Heath et al., 1989).

Retinal degeneration has been reported in cats treated with high doses (20 mg/kg every 24 hours) of enrofloxacin (Wiebe and Hamilton, 2002). Vision may or may not return after enrofloxacin therapy is discontinued. Although the exact mechanism of retinal degeneration in cats is unknown, it appears that a similar retinal degeneration can be reproduced from either direct intravitreal injection of high concentrations of enrofloxacin or exposure to UVA light and enrofloxacin in laboratory animals. The fluoroquinolone molecular structure is similar structurally to other

drugs known to directly induce retinal degeneration. Experimental evidence suggests that both enrofloxacin and its breakdown products induce retinal degeneration. Development of retinal degeneration also depends on the maximum concentration of enrofloxacin and/or its metabolites accumulating in the retina over time. Risk factors for cats appear to include: (1) high doses resulting in high plasma concentrations of enrofloxacin, (2) rapid IV administration, (3) chronic treatment, and (4) advanced age. Other factors may include: (1) prolonged exposure to UVA light while on enrofloxacin therapy, (2) drug interactions, and (3) altered metabolism or reduced elimination resulting in drug accumulation. Because of this, it has been recommended that administration of high doses of all fluoroquinolones be avoided in the cat whenever possible. However, this toxicity may be fluoroquinolone-dependent, as limited manufacturer studies with marbofloxacin, orbifloxacin and pradofloxacin did not demonstrate ocular toxicity in cats.

Neurotoxic effects causing central nervous system disturbances (seizures, dizziness, ataxia, insomnia, restlessness, somnolence, tremors) are common adverse effects of fluoroquinolones in humans and have been reported in horses, dogs, and cats treated with enrofloxacin. Rapid IV administration of high doses of enrofloxacin to horses causes transient neurological signs, including excitability and seizure-like activity. The adverse CNS effects are due to GABA receptor antagonism, and are usually dose and specific fluoroquinolone dependent. Enrofloxacin has been associated with increased frequency and intensity of seizures in epileptic dogs (Van Cutsem et al., 1990). Because of greater penetration of the blood-brain barrier than ciprofloxacin, enrofloxacin causes hallucinations when administered to humans.

Photosensitivity and Achilles tendon rupture has been associated with the use of fluoroquinolones in humans but has not been reported in animals. Occasionally, mild interstitial inflammation of the kidney tubular walls has been associated with precipitation of fluoroquinolone complexes. Crystalluria leading to obstructive uropathy has been reported in human studies, but it is uncommon. Other renal toxicities may include acute renal failure associated with interstitial nephritis. However, in human medicine, most cases of renal toxicity have been associated with overdoses.

The popularity of fluoroquinolones for use in dogs

Table 17.7. Usual dosages of fluoroquinolones in animals.^a

Drug	Species	Route	Dose range (mg/kg)	Interval (hrs)	Comments ^b
Enrofloxacin	Dogs	PO, IV	5.0 - 20	12 - 24	15 to 20 minute infusion
		IM	2.5	24	
	Cats	PO, IV	5.0	12	15 to 20 minute infusion
		IM	2.5		
	Cattle	SC	2.5 - 5.0	24 for 3-5 days	
			7.5-12.5	Once	
	Horses ^b	IV	5.0	24	Slow IV bolus
		PO	7.5	24	
	Pigs ^c	IM	2.5-7.5	24	
Orbifloxacin	Dogs, cats	PO	2.5 - 7.5	24	
Difloxacin	Dogs	PO	5-10	24	
Ciprofloxacin	Dogs	PO	11-23	12	
Marbofloxacin	Dogs, cats	PO	2.75-5.5	12	
Danofloxacin	Calves	SC	6.0	48 for 2 doses	IV should be administered as a 15 to 20 minute infusion

^aSources are from drug sponsor, package insert, or published data as indicated.

^bFluoroquinolones may cause arthropathies in juvenile animals.

^cNot for use in pigs that may enter the human food chain in the United States.

has been associated with the emergence of canine toxic shock syndrome and necrotizing fascitis caused by *Streptococcus canis* (Miller et al., 1996). Minor infections caused by *S. canis* have developed into very severe illness in dogs treated with fluoroquinolone monotherapy. Enrofloxacin can cause a bacteriophage-induced lysis of *S. canis* and superantigen expression (Ingrey et al., 2003). Superantigens are powerful inducers of T-cell proliferation, causing the release of massive amounts of host cytokines with potentially lethal effects. The septic shock syndrome can be exacerbated by the concurrent use of corticosteroids or nonsteroidal anti-inflammatory drugs.

Administration and Dosage

Fluoroquinolones are usually administered orally in small animals, horses, or preruminants, and parenterally (IV, IM, and SC) in ruminants. The usual dosages of some currently available fluoroquinolones are shown in Table 17.7.

Perhaps more than with any other class of antimicrobial agent, dosage of fluoroquinolones should be based on the susceptibility of the bacterial target. Clinical efficacy of the fluoroquinolone is dependent on dose and bacterial pathogen. To maximize clinical

efficacy and reduce selection of resistant bacteria, C_{\max} :MIC ratios $\geq 10:1$ or AUC_{0-24} :MIC ratios $\geq 125:1$ are recommended.

There is an argument as to whether it is the C_{\max} :MIC or the AUC_{0-24} :MIC ratio that determines maximum clinical efficacy. While these two parameters are closely related, there are subtle differences. For example, when administering a fluoroquinolone at 12 hour dosing intervals, the C_{\max} after the first and second dose will be essentially the same, as will the AUC_{0-12} . However, since it is the AUC_{0-24} that is important, when a drug is administered every 12 hours in a 24 hour period the AUC value is doubled.

Since the fluoroquinolones exhibit concentration-dependent killing, this uncertainty as to whether C_{\max} or AUC_{0-24} is most important can be addressed by knowledge of the MIC of the bacterial pathogen. For pathogens with low MICs, i.e., ≤ 0.06 , AUC_{0-24} :MIC ratios are important in determining clinical efficacy; for pathogens with high but still susceptible MICs, since there is an increased chance of selecting for resistant organisms, the C_{\max} :MIC ratio may be the better predictor of clinical efficacy because of the concentration killing effect (Craig and Dalhoff, 1998). Low AUC_{0-24} :MIC ratios, even if found to be clinically effective, can still contribute to the selection of resistant organisms (Thomas et al., 1998).

Designing appropriate and optimal dosing regimens for fluoroquinolones requires: (1) knowledge of the selected agents' C_{\max} or AUC_{0-24} within the recommended dosing range; (2) knowledge of the MIC of the selected fluoroquinolone for the pathogen; and (3) understanding pharmacodynamic indices. Failure to apply these three principles results in inappropriate fluoroquinolone use, which in turn contributes to the selection of resistant organisms. The emergence of fluoroquinolone resistance diminishes the longevity of these drugs for use in veterinary medicine.

Use of the C_{\max} :MIC Ratio Approach

If the MIC is known, the peak serum concentration should be 10 times the MIC, i.e., the ratio of C_{\max} :MIC is $\geq 10:1$. For example, if the MIC is 0.12 $\mu\text{g/ml}$, 10 times that would require a peak serum concentration of at least 1.2 $\mu\text{g/ml}$. From Table 17.5 it can be seen that enrofloxacin produces a serum C_{\max} of approximately 1.4 $\mu\text{g/ml}$ when administered at 5 mg/kg to dogs. This is similar to the serum C_{\max} for marbofloxacin and orbifloxacin when administered at doses of 2 and 2.5 mg/kg, respectively. If the MIC of a pathogen is 0.25 $\mu\text{g/ml}$, a higher dose (from Table 17.5) of 10 mg/kg enrofloxacin, 4 mg/kg marbofloxacin, or 5 mg/kg orbifloxacin would be required to exceed the desired C_{\max} :MIC ratio of 10:1. This is assuming there is a doubling of the serum C_{\max} when the dose of a fluoroquinolone is doubled.

Use of the AUC_{0-24} :MIC Ratio Approach

For this approach, relate the AUC_{0-24} to the MIC. For example, if the MIC for enrofloxacin is 0.06 $\mu\text{g/ml}$, the required dose is between 2.75 mg/kg and 5 mg/kg. (Table 17.5 illustrates that when enrofloxacin is administered at a dose of 5 mg/kg, the AUC_{0-24} is 8.74. Therefore, 8.74 divided by the MIC of 0.06 $\mu\text{g/ml}$ results in an AUC_{0-24} :MIC ratio of 145.) These ratios have been shown to maximize clinical efficacy and minimize selection of resistant organisms, since a ratio of $\geq 125:1$ is required for optimal bactericidal action (Figs. 17.4 and 17.5) (Thomas et al., 1998; Forrest et al., 1993). Figure 17.4 illustrates the relationship between AUC_{0-24} :MIC and the probability of selecting for resistant organisms. Figure 17.5 depicts the relationship between the AUC_{0-24} :MIC of ciprofloxacin and eradication of the pathogen.

In using these approaches, the dosing interval must prevent serum concentrations from dropping below

the MIC of the pathogen for more than 20% of the dosing interval. This is not usually a problem when AUC_{24} :MIC ratios are used.

Clinical Applications

Fluoroquinolones in veterinary use offer the advantages of oral administration in many species, high potency against many Gram-negative aerobic pathogens, moderate activity against Gram-positive aerobes, widespread distribution throughout the body, and low toxicity. Their disadvantages include the tendency to select for resistant bacteria if dosed inappropriately and their only moderate activity against Gram-positive aerobes, such as pyogenic streptococci (e.g., *Streptococcus canis*). They are very effective in the treatment of urinary tract infections in animals and can be useful for serious infections such as septicemia and pneumonia caused by Gram-negative bacteria (*E. coli*, *Pasteurella* spp.), for the treatment of skin and many soft-tissue infections caused by Gram-negative or some Gram-positive aerobic bacteria, and for intra-abdominal infections caused by Gram-negative aerobes. Human ophthalmic formulations are routinely used to treat Gram-negative infectious keratitis. Fluoroquinolones are the most effective antimicrobial agents for the treatment of chronic bacterial prostatitis caused by susceptible Gram-negative bacteria. They are effective in the treatment of *Mycoplasma* infections in some species. Because of their potency and ability to enter phagocytes, they have the potential to be valuable for the treatment of infections caused by atypical bacteria, including mycobacteria, *Brucella* spp., *Chlamydia/Chlamydophila* spp., *Coxiella* spp., *Ehrlichia* spp., and *Rickettsia* spp. However, documentation is required in veterinary medicine of their efficacy for treating infections caused by many of these pathogens.

The introduction of fluoroquinolones for companion animals was associated with their promotion as drugs of choice for numerous infectious disease processes. One justification was that plasmid-mediated resistance was not likely to occur, or if it did, it would not be transferable. However, since the introduction of these drugs into clinical medicine, plasmid-mediated resistance has been described (Martinez-Martinez, 1998; Wang, 2003). Unless they are used with optimal dosing strategies, the fluoroquinolones may soon be ineffective in treating anything but the simplest infec-

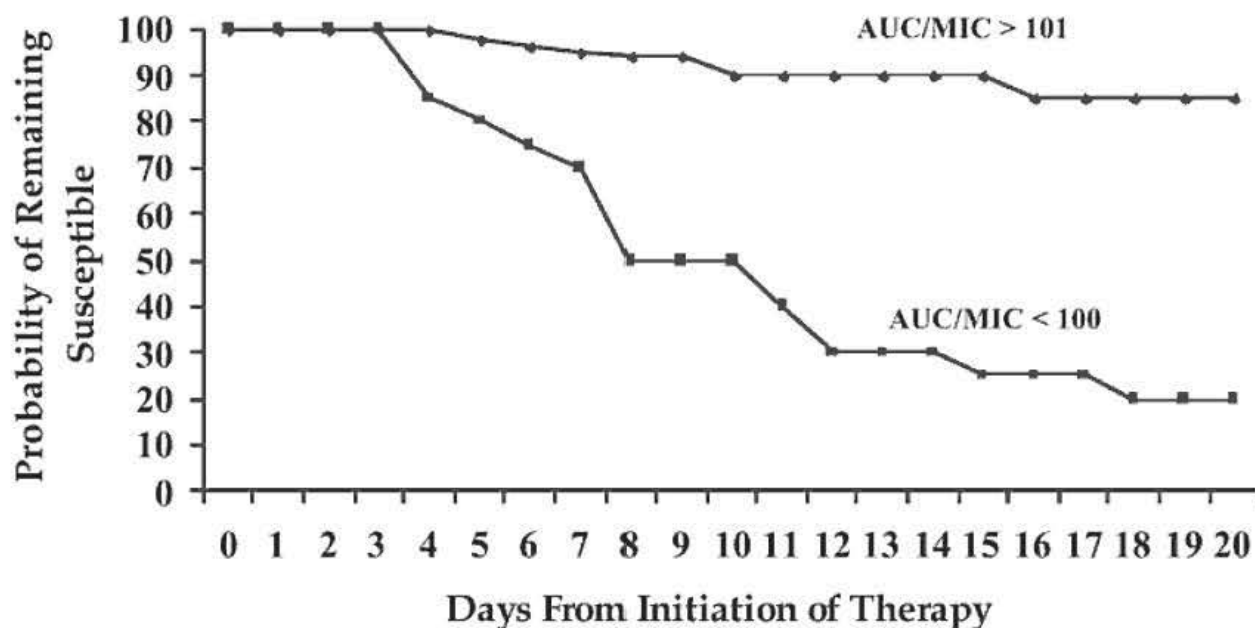


Figure 17.4. Relationship between $AUC_{0-24}:MIC$ and the probability of selecting for resistant bacteria (in humans). Thomas et al. (1998); reproduced with permission.

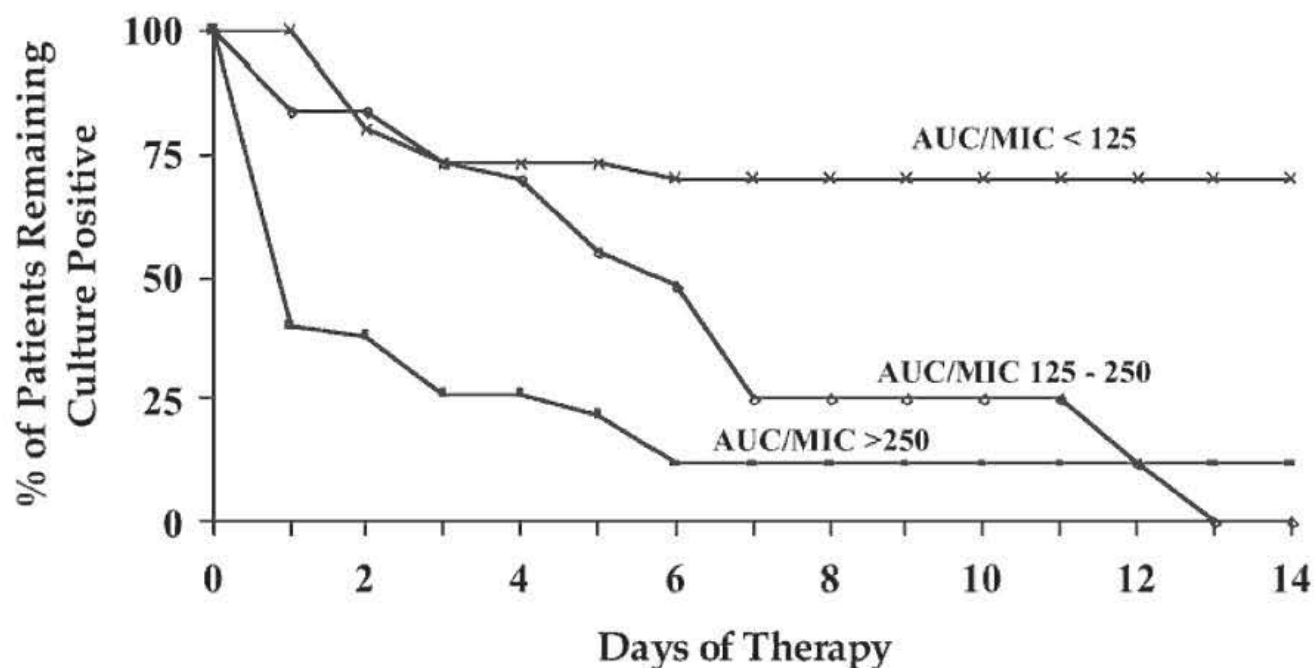


Figure 17.5. Time (days of therapy) to bacterial eradication (in humans) versus $AUC_{0-24}:MIC$ ratio: a time-to-event (survival) plot. Forrest et al. (1993); reproduced with permission.

tions, despite the promise they offered when they were first introduced.

Cattle, Sheep and Goats

Fluoroquinolones are quite active when tested against bacteria associated with acute respiratory disease in cattle, sheep, and goats, such as *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*. They also have the potential to be effective against several other species of bacteria known to cause disease in these animals, especially Gram-negative bacteria such as *E. coli* and *Salmonella*, although the MICs of these pathogens will most likely be higher than the MICs of the bacteria associated with acute respiratory disease, thus requiring higher doses and longer withdrawal times. Other indications include mastitis, metritis, conjunctivitis, and infections caused by *Mycoplasma*, such as pneumonia and otitis media. The MICs for enrofloxacin and danofloxacin are within the same range as the respiratory pathogens for which these drugs are approved (Rosenbusch et al., 2005). There is some evidence of efficacy for otitis media in calves (Francoz et al., 2004), but prolonged therapy is required.

Although fluoroquinolones should be effective in treating most of the above indications, in the US enrofloxacin and danofloxacin are approved only for the treatment of acute pneumonia in beef cattle, and veterinarians are legally prohibited from any extra-label use of fluoroquinolones in food animals. Extra-label use includes any alteration of dose, frequency, or duration in any animal that may enter the human food chain. In Canada, both drugs are approved and there are cautions against extra-label drug use on product labeling, but there is no legal prohibition against it. In other countries, enrofloxacin, danofloxacin, and marbofloxacin have a variety of approvals for the treatment of bovine respiratory disease, colibacillosis and mastitis in lactating dairy cattle. Treatment regimens vary among products, but all should be dosed according to the ideal pharmacokinetic/pharmacodynamic methods described in this chapter. Injectable formulations tend to be irritating to muscle tissues, so most products are labeled for SC injection.

Swine

Fluoroquinolones have established value in the treatment of *Mycoplasma hyopneumoniae* infections and have the potential for the prevention or treatment of *Actinobacillus pleuropneumoniae*, *Escherichia coli*, and

Pasteurella multocida infections. Their use should be optimized to the individual pathogen and infection. Fluoroquinolones should never be administered in feed because residues can contaminate the environment from the feed mill to the farm. Because of concern about resistance in zoonotic foodborne pathogens, fluoroquinolones are prohibited from use in pigs in the United States. Several fluoroquinolone products are approved for use in swine in other countries to treat respiratory disease and Metritis-Mastitis-Agalactia syndrome.

Horses

Because fluoroquinolones can be administered orally, they are useful in horses for the treatment of a variety of Gram-negative infections caused by susceptible bacteria resistant to alternative, first-choice drugs. For example, enrofloxacin was used in the successful treatment of chronic *E. coli* pleuritis caused by an otherwise resistant organism (Heath et al., 1989). Kaartinen et al. (1997) found IM administration to be very irritating, resulting in swelling or tenderness at the injection site with elevated creatine kinase activity for up to 32 hours after injection. Cattle formulations can be administered slowly IV (Bertone et al., 2000) or formulated into a gel for oral administration (Epstein et al., 2004). Because of the potential of fluoroquinolones to cause cartilage erosion, their use is not recommended in young, growing horses.

Dogs and Cats

Fluoroquinolones have provided small-animal clinicians with a truly exciting new class of antimicrobials. Never before have they had products with such a broad-spectrum of activity as the fluoroquinolones combined with the pharmacokinetic properties that allow for oral administration on a once-a-day basis. This has allowed clinicians to treat a larger number of patients as outpatients with more assurance of owner compliance. In most countries, only enrofloxacin is available as an injectable product. Intramuscular or SC injections are irritating but the product can be safely administered slowly IV. Enrofloxacin, difloxacin, ibafloxacin, marbofloxacin, pradofloxacin, and orbifloxacin are available for oral use in small animals in many countries. Human formulations of ciprofloxacin can be used as long as the dose is corrected for bioavailability (33% in cats, 50% in dogs).

Because fluoroquinolones can penetrate nearly

every tissue in the body, these drugs can be used to treat infections such as prostatitis and mastitis caused by susceptible bacteria; respiratory infections including rhinitis and pneumonia, including those caused by *Bordetella bronchiseptica*; deep and superficial pyoderma, otitis media and externa, and wound infections caused by susceptible organisms; peritonitis (used in combination with metronidazole if anaerobic bacteria are suspected); osteomyelitis caused by susceptible Gram-negative aerobes; and infections caused by mycoplasmas such as rhinitis/conjunctivitis and soft-tissue infections. At therapeutic doses the fluoroquinolones have proven to be relatively safe with few reported side effects. If adverse reactions do occur, they are not as frequent as those reported in human medicine. The fluoroquinolones are not recommended for administration to animals less than eight months of age or to large-breed dogs less than 18 months of age. However, since their approval in the 1980s, they have been used to treat life-threatening infections in young dogs and cats without any published reports of arthropathic effects.

Poultry

In intensive poultry production, rapidly acting antimicrobial agents are needed in the face of explosive outbreaks of infectious disease. The most critical of such infections is *E. coli* septicemia and cellulitis (see Chapter 35), but other important Gram-negative aerobic infections include salmonellosis, *Haemophilus paragallinarum*, and *Pasteurella multocida* (Bauditz, 1987). Two fluoroquinolones, sarafloxacin and enrofloxacin, were developed for poultry use and approved as water medication. While studies have shown that the treatment of colibacillosis with enrofloxacin does not cause significant increases in resistant *E. coli* (van Boven et al., 2003), there is evidence that this treatment selects for ciprofloxacin-resistant *Campylobacter* in chickens (McDermott et al., 2002; Luo et al., 2003; Humphrey et al., 2005).

In the United States, the approvals of both enrofloxacin and sarafloxacin have been withdrawn because of fears that fluoroquinolone-resistant *Campylobacter* from poultry contribute to human foodborne illness. In Canada, an egg dip solution for the treatment of salmonellosis in turkey eggs was once available but has been withdrawn from the market. Oral formulations of enrofloxacin and sarafloxacin have never been approved for Canadian poultry. Many of

the veterinary fluoroquinolones are approved and continue to be administered orally to poultry in other countries.

Bibliography

- Abadia AR, et al. 1995. Ciprofloxacin pharmacokinetics in dogs following oral administration. *Zentralbl Veterinarmed A* 42:505.
- Abadia AR, et al. 1994. Disposition of ciprofloxacin following intravenous administration in dogs. *J Vet Pharmacol Ther* 17:384.
- Albarells GA, et al. 2004. Pharmacokinetics of ciprofloxacin after single intravenous and repeat oral administration to cats. *J Vet Pharmacol Ther* 27:155.
- Albarells GA, et al. 2005. Pharmacokinetics of marbofloxacin after single intravenous and repeat oral administration to cats. *Vet J* 170:222.
- Alekshun MN, Levy SB. 1999. The *mar* regulon: multiple resistance to antibiotics and other toxic chemicals. *Trends in Microbiol* 7:410.
- Ball P. 2000. Quinolone generations: natural history or natural selection. *J Antimicrob Chemother* 46 (Suppl T1):17.
- Bauditz R. 1987. Results of clinical studies with Baytril in poultry. *Vet Med Rev* 2:122.
- Bebear CM, et al. 2003. DNA gyrase and topoisomerase IV mutations in clinical isolates of *Ureaplasma* spp. and *Mycoplasma hominis* resistant to fluoroquinolones. *Antimicrob Agents Chemother* 47:3323.
- Birmingham EC, et al. 2000. Pharmacokinetics of enrofloxacin administered intravenously and orally to foals. *Am J Vet Res* 61:706.
- Bertone AL, et al. 2000. Effect of long-term administration of an injectable enrofloxacin solution on physical and musculoskeletal variables in adult horses. *J Am Vet Med Assoc* 217:1514.
- Bryskier A. 2005. Fluoroquinolones. In Bryskier A, ed. *Antimicrobial Agents: Antibacterials and Antifungals*. ASM Press, pp 668.
- Brown SA. 1996. Fluoroquinolones in animal health. *J Vet Pharm Ther* 19:1.
- Burkhardt JE, et al. 1992. Ultrastructural changes in articular cartilages of immature beagle dogs dosed with difloxacin, a fluoroquinolone. *Vet Pathol* 29:230.
- Carbone M, et al. 2001. Activity and postantibiotic effect of marbofloxacin, enrofloxacin, difloxacin and ciprofloxacin against feline *Bordetella bronchiseptica* isolates. *Vet Microbiol* 81:79.
- Cohen SP, et al. 1988. *marA* locus causes decreased expression of OmpF porin in multiple-antibiotic-resistant (Mar) mutants of *Escherichia coli*. *J Bacteriol* 170:5416.
- Cohen SP, et al. 1993. A multidrug resistance regulatory chromosomal locus is widespread among enteric bacteria. *J Infect Dis* 168:484.
- Coulet M, et al. 2002. In vitro and in vivo pharmacodynamic properties of the fluoroquinolone ibafloxacin. *J Vet Pharmacol Ther* 25:401.

- Coulet M, et al. 2002. Pharmacokinetics of ibafloxacin following intravenous and oral administration to healthy Beagle dogs. *J Vet Pharmacol Ther* 25:89.
- Coulet M, et al. 2005. Pharmacokinetics of ibafloxacin in healthy cats. *J Vet Pharmacol Ther* 28:37.
- Craig WA, Dalhoff A. 1998. Pharmacodynamics of fluoroquinolones in experimental animals. In: Kuhlman J, et al., eds. *Quinolone Antibacterials*, New York: Springer-Verlag.
- de Jong A, et al. 2004. Antibacterial activity of pradofloxacin against canine and feline pathogens isolated from clinical cases. Abstract 2nd International Conference on Antimicrobial Agents in Veterinary Medicine, Ottawa, Canada.
- Dowling PM, et al. 1995. Pharmacokinetics of ciprofloxacin in ponies. *J Vet Pharmacol Ther* 18:7.
- Eliopoulos GM, Moellering RC, Jr. 1996. Antimicrobial combinations, Chapter 9. In: Lorian V ed. *Antibiotics in Laboratory Medicine*, 4th ed. Baltimore: Williams & Wilkins.
- Epstein K, et al. 2004. Pharmacokinetics, stability, and retrospective analysis of use of an oral gel formulation of the bovine injectable enrofloxacin in horses. *Vet Ther* 5:155.
- Everett MJ, et al. 1996. Contributions of individual mechanisms to fluoroquinolone resistance in 36 *Escherichia coli* strains isolated from humans and animals. *Antimicrob Agents Chemother* 40:2380.
- Ewert K. 1997. Pharmacokinetics and in vitro antimicrobial activity of enrofloxacin. *Am Assoc Swine Practitioners*, pp 153.
- Federal Register. 2000. 65 Fed Reg 64954 (Oct 31, 2000).
- Ferrero L, et al. 1994. Cloning and primary structure of *Staphylococcus aureus* DNA topoisomerase IV: a primary target of fluoroquinolones. *Mol Microbiol* 13:641.
- Forrest A, et al. 1993. Pharmacodynamics of intravenous ciprofloxacin in seriously ill patients. *Antimicrob Agents Chemother* 37:1073.
- Francoz D, et al. 2004. Otitis media in dairy calves: a retrospective study of 15 cases (1987 to 2002). *Can Vet J* 45:661.
- Ganiere JP, et al. 2005. Antimicrobial drug susceptibility of *Staphylococcus intermedius* clinical isolates from canine pyoderma. *J Vet Med B Infect Dis Vet Public Health* 52:25.
- Ganiere JP, et al. 2004. In vitro antimicrobial activity of orbifloxacin against *Staphylococcus intermedius* isolates from canine skin and ear infections. *Res Vet Sci* 77:67.
- Giguère S, et al. 1996. Pharmacokinetics of enrofloxacin in adult horses and concentration of the drug in serum, body fluids, and endometrial tissues after repeated intragastrically administered doses. *Am J Vet Res* 57:1025.
- Giguère S, Bélanger M. 1997. Concentration of enrofloxacin in equine tissues after long term oral administration. *J Vet Pharm Ther* 20:402.
- Goldstein EJC, et al. 1998. Trovafloxacin compared with levofloxacin, ofloxacin, ciprofloxacin, azithromycin and clarithromycin against unusual aerobic and anaerobic human and animal bite-wound pathogens. *J Antimicrob Chemother* 41:391.
- Gould IL, et al. 1990. Concentration-dependent bacterial killing, adaptive resistance and post-antibiotic effect of ciprofloxacin alone and in combination with gentamicin. *Drugs Exper Clin Res* 26:621.
- Grohe K. 1998. The chemistry of the quinolones: methods of synthesizing the quinolone ring system. In: Kuhlman J, et al. eds. *Quinolone Antibacterials*. New York:Springer-Verlag.
- Guthrie RM, et al. 2004. Treating resistant respiratory infections in the primary care setting: the role of the new quinolones. University of Cincinnati College of Medicine Continuing Medical Education.
- Heath SE, et al. 1989. Chronic pleuritis in a horse. *Can Vet J* 30:69.
- Heinen E. 2002. Comparative serum pharmacokinetics of the fluoroquinolones enrofloxacin, difloxacin, marbofloxacin, and orbifloxacin in dogs after single oral administration. *J Vet Pharmacol Ther* 25:1.
- Hoshino K, et al. 1994. Comparison of inhibition of *Escherichia coli* topoisomerase IV by quinolones with DNA gyrase inhibition. *Antimicrob Agents Chemother* 38:2623.
- Humphrey TJ, et al. 2005. Prevalence and subtypes of ciprofloxacin-resistant *Campylobacter* spp. in commercial poultry flocks before, during, and after treatment with fluoroquinolones. *Antimicrob Agents Chemother* 49:690.
- Ingrey KT, et al. 2003. A fluoroquinolone induces a novel mitogen-encoding bacteriophage in *Streptococcus canis*. *Infect Immun* 71:3028.
- Intorre L, et al. 1995. Enrofloxacin-theophylline interaction: influence of enrofloxacin on theophylline steady-state pharmacokinetics in the beagle dog. *J Vet Pharmacol Ther* 18:352.
- Jacoby GA, et al. 2003. Prevalence of plasmid-mediated quinolone resistance. *Antimicrob Agents Chemother* 47:559.
- Kaartinen L, et al. 1997. Pharmacokinetics of enrofloxacin in horses after single intravenous and intramuscular administration. *Equine Vet J* 29:378.
- Kaatz GW, Seo SM. 1998. Topoisomerase mutations in fluoroquinolone-resistant and methicillin-susceptible and resistant clinical isolates of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 42:197.
- Kadlec K, et al. 2004. Antimicrobial susceptibility of *Bordetella bronchiseptica* isolates from porcine respiratory tract infections. *Antimicrob Agents Chemother* 48:4903.
- Kato J, et al. 1990. New topoisomerase essential for chromosome segregation in *E. coli*. *Cell* 63:393.
- Kato J, et al. 1992. Purification and characterization of DNA topoisomerase IV in *Escherichia coli*. *J Biol Chem* 267:25676.
- Khodursky AB, et al. 1995. Topoisomerase IV is a target of quinolones in *Escherichia coli*. *Proc Natl Acad Sci US* 92:11801.
- Kung K, et al. 1993. Pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin after intravenous and oral administration of enrofloxacin in dogs. *J Vet Pharmacol Ther* 16:462.
- Lautzenhiser SJ, et al. 2001. In vitro antibacterial activity of enrofloxacin and ciprofloxacin in combination against *Escherichia coli* and staphylococcal clinical isolates from dogs. *Res Vet Sci* 70:239.

- Luo N, et al. 2003. In vivo selection of *Campylobacter* isolates with high levels of fluoroquinolone resistance associated with *gyrA* mutations and the function of the CmeABC efflux pump. *Antimicrob Agents Chemother* 47:390.
- Martinez-Martinez, et al. 1998. Quinolone resistance from a transferable plasmid. *Lancet* 351:797.
- Martinez M, et al. 2005. Pharmacology of the Fluoroquinolones: A perspective for the use in domestic animals. *Vet J*. (In press).
- Maxwell A, Critchlow SE. 1998. Mode of action. In: Kuhlmann J, et al., eds. *Quinolone Antibacterials*. New York: Springer-Verlag, p 119.
- McDermott PF, et al. 2002. Ciprofloxacin resistance in *Campylobacter jejuni* evolves rapidly in chickens treated with fluoroquinolones. *J Infect Dis* 185:837.
- Meunier D, et al. 2004. A seven-year survey of susceptibility to marbofloxacin of pathogenic strains isolated from pets. *Int J Antimicrob Agents* 24:592.
- Meunier D, et al. 2004. Seven years survey of susceptibility to marbofloxacin of bovine pathogenic strains from eight European countries. *Int J Antimicrob Agents* 24:268.
- Miller CW, et al. 1996. Streptococcal toxic shock in dogs. *J Am Vet Med Assoc* 209:1421.
- Munoz R, De La Campa AG. 1996. ParC subunit of DNA topoisomerase IV of *Streptococcus pneumoniae* is a primary target of fluoroquinolones and cooperates with DNA gyrase A subunit in forming resistance phenotype. *Antimicrob Agents Chemother* 40:2252.
- Nakamura S, et al. 1989. *gyrA* and *gyrB* mutations in quinolone-resistant strains of *Escherichia coli*. *Antimicrob Agents Chemother* 33:254.
- Ng EY, et al. 1996. Quinolone resistance mutations in topoisomerase IV: relationship to the *flqA* locus and genetic evidence that topoisomerase IV is the primary target and DNA gyrase is the secondary target of fluoroquinolones in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 40:1881.
- Nielsen P, Gyrd-Hansen N. 1997. Bioavailability of enrofloxacin after oral administration to fed and fasted pigs. *Pharmacol Toxicol* 80:246.
- Nikaido H, Vaara M. 1985. Molecular basis of bacterial outer membrane permeability. *Microbiol Rev* 49:1.
- Okusu H, et al. 1996. AcrAB efflux pump plays a major role in the antibiotic resistance phenotype of *Escherichia coli* multiple-antibiotic-resistance (Mar) mutants. *J Bacteriol* 178:306.
- Oethinger M, et al. 2000. Ineffectiveness of topoisomerase mutations in mediating clinically significant fluoroquinolone resistance in *Escherichia coli* in the absence of the AcrAB efflux pump. *Antimicrob Agents Chemother* 44:10.
- Piddock LJ, et al. 1998. Role of mutation in the *gyrA* and *parC* genes of nalidixic-acid-resistant salmonella serotypes isolated from animals in the United Kingdom. *J Antimicrob Chemother* 41:635.
- Poole K. 2000. Efflux-mediated resistance to fluoroquinolones in gram-negative bacteria. *Antimicrob Agents Chemother* 44:2233.
- Post LO, et al. 2003. The effect of endotoxin and dexamethasone on enrofloxacin pharmacokinetic parameters in swine. *J Pharmacol Exp Ther* 304:889.
- Preston SL, et al. 1998. Levofloxacin population pharmacokinetics and creation of a demographic model for prediction of individual drug clearance in patients with serious community-acquired infection. *Antimicrob Agents Chemother* 42:1098.
- Rosenbusch RF, et al. 2005. In vitro antimicrobial inhibition profiles of *Mycoplasma bovis* isolates recovered from various regions of the United States from 2002-2003. *J Vet Diagn Invest* 17:436.
- Schentag JJ, Scully BE. 1999. Quinolones. In: Yu VL, et al (eds.). *Antimicrobial Therapy and Vaccines*. New York: Williams & Wilkins, p 875.
- Schentag JJ, et al. 2003. Fluoroquinolone AUC breakpoints and the link to bacterial killing rates, Part 2: human trials. *Ann Pharmacother* 37:1478.
- Seguin MA, et al. 2004. Pharmacokinetics of enrofloxacin in neonatal kittens. *Am J Vet Res* 65:350.
- Schneider M, et al. 1996. Pharmacokinetics of marbofloxacin in dogs after oral and parenteral administration. *J Vet Pharmacol Ther* 19:56.
- Schneider M, et al. 2004. Pharmacokinetics of marbofloxacin in lactating cows after repeated intramuscular administrations and pharmacodynamics against mastitis isolated strains. *J Dairy Sci* 87:202.
- Speakman AJ, et al. 1997. Antimicrobial susceptibility of *Bordetella bronchiseptica* isolates from cats and a comparison of the agar dilution and E-test methods. *Vet Microbiol* 54:63.
- Speakman AJ, et al. 2000. Antibiotic susceptibility of canine *Bordetella bronchiseptica* isolates. *Vet Microbiol* 71:193.
- Stein GE. 1988. The 4-quinolone antibiotics: past, present and future. *Pharmacother* 8:301.
- Takiff HE, et al. 1994. Cloning and nucleotide sequence of *Mycobacterium tuberculosis gyrA* and *gyrB* genes and detection of quinolone resistance mutations. *Antimicrob Agents Chemother* 38:773.
- Taylor DE, Chau AS. 1997. Cloning and nucleotide sequence of the *gyrA* gene from *Campylobacter fetus* subsp. *fetus* ATCC 27374 and characterization of ciprofloxacin-resistant laboratory and clinical isolates. *Antimicrob Agents Chemother* 41:665.
- TerHune TN, et al. 2005. Comparison of pharmacokinetics of danofloxacin and enrofloxacin in calves challenged with *Mannheimia haemolytica*. *Am J Vet Res* 66:342.
- Thomas JK, et al. 1998. Pharmacodynamic evaluation of factors associated with the development of bacterial resistance in acutely ill patients during therapy. *Antimicrob Agents Chemother* 42:521.
- Tran JH, Jacoby GA. 2002. Mechanism of plasmid-mediated quinolone resistance. *Proc Natl Acad Sci USA* 99:5638.
- Van Bambeke F, et al. 2005. Quinolones in 2005: an update. *Clin Microbiol Infect Dis* 11:256.
- Van Boven M, et al. 2003. Rapid selection of quinolone resistance in *Campylobacter jejuni* but not in *Escherichia coli* in individually housed broilers. *J Antimicrob Chemother* 52:719.

- Van Cutsem PM, et al. 1990. The fluoroquinolone antimicrobials: structure, antimicrobial activity, pharmacokinetics, clinical use in domestic animals and toxicity. *Cornell Vet* 80:173.
- Van den Hoven R, et al. 2000. In vitro activity of difloxacin against canine bacterial isolates. *J Vet Diagn Invest* 12:218.
- Varma R, et al. 2003. Pharmacokinetics of enrofloxacin and its active metabolite ciprofloxacin in cows following single dose intravenous administration. *J Vet Pharmacol Ther* 26:303.
- Vila J, et al. 1996. Detection of mutations in *parC* in quinolone-resistant clinical isolates of *Escherichia coli*. *Antimicrob Agents Chemother* 40:491.
- Vivrette SL, et al. 2001. Quinolone-induced arthropathy in neonatal foals. 47th Annual American Association of Equine Practitioners Convention; 376.
- Walker RD, Thornsberry C. 1998. Decrease in antibiotic susceptibility or increase in resistance. *J Antimicrob Chemother* 41:1.
- Walker RD, et al. 1990. Serum and tissue cage fluid concentrations of ciprofloxacin after oral administration of the drug to healthy dogs. *Am J Vet Res* 51:896.
- Walker RD, et al. 1992. Pharmacokinetic evaluation of enrofloxacin administered orally to healthy dogs. *Am J Vet Res* 53:2315.
- Wang M, et al. 2003. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. *Antimicrob Agents Chemother* 48:2242.
- Wang M, et al. 1993. Cloning and nucleotide sequence of the *Campylobacter jejuni gyrA* gene and characterization of quinolone resistance mutations. *Antimicrob Agents Chemother* 37:457.
- Watts JL, et al. 1997. In vitro activity of premafloxacin, a new extended-spectrum fluoroquinolone, against pathogens of veterinary importance. *Antimicrob Agents Chemother* 41:1190.
- Wiebe V, Hamilton P. 2002. Fluoroquinolone-induced retinal degeneration in cats. *J Am Vet Med Assoc* 221:1568.
- Yoon JH, et al. 2004. The effect of enrofloxacin on cell proliferation and proteoglycans in horse tendon cells. *Cell Biol Toxicol* 20:41.
- Yoshida H, et al. 1990. Quinolone resistance-determining region in the DNA gyrase *gyrA* gene of *Escherichia coli*. *Antimicrob Agents Chemother* 34:1271.
- Yoshida H, et al. 1988. Quinolone-resistant mutations of the *gyrA* gene of *Escherichia coli*. *Mol Gen Genetics* 211:1.
- Yoshimura H, et al. 2002. Comparative in vitro activity of 16 antimicrobial agents against *Actinobacillus pleuropneumoniae*. *Vet Res Commun* 26:11.
- Zechiedrich EL, Cozzarelli NR. 1995. Roles of topoisomerase IV and DNA gyrase in DNA unlinking during replication in *Escherichia coli*. *Genes Develop* 9:2859.

Miscellaneous Antimicrobials: Ionophores, Nitrofurans, Nitroimidazoles, Rifamycins, Oxazolidinones, and Others

Patricia M. Dowling

This chapter examines in detail a variety of minor antimicrobial classes used in veterinary medicine—the ionophores, nitrofurans, nitroimidazoles, rifampin, and oxazolidinones—and briefly comments on other antimicrobials, including arsenicals, carbadox, fusidic acid, isoniazid, methenamine, and novobiocin.

Ionophore Antibiotics

Carboxylic ionophore polyether antibiotics are *Streptomyces* products used in agriculture primarily for feed efficiency and anticoccidial activity. They behave as an alkali metal ionophore in altering bacterial cell permeability, complexing with sodium in the cell membrane to cause passive extracellular transport of potassium ions and replacement by hydrogen ions, which kills the cell by lowering intracellular pH. By selectively affecting Gram-positive organisms, ionophore antibiotics cause rumen microflora to shift toward a more Gram-negative population. This increases propionic acid production while decreasing production of acetic and butyric acid. This shift in volatile fatty acids promotes increased feed efficiency. In the absence of ionophores, ruminal sugars are metabolized to acetic acid and butyric acid and lose some of their potential energy in the form of carbon dioxide and methane. However, when these sugars are converted to propionic acid, losses are reduced and energy content per unit of feed consumed is increased (Bergen and Bates, 1984). Not all bacteria are susceptible to ionophores, and some species have several mechanisms of ionophore resistance. The prophylactic use of antimicrobials as growth promotants in food animals has fallen under greater scrutiny due to concerns over antimicrobial resistance. But because of the complexity and high degree of spe-

cificity of ionophore resistance, it appears that ionophores do not contribute to the development of antimicrobial resistance to important human drugs, and there is thus no need to eliminate them from use in animal feeds (Callaway et al., 2003).

Pharmacokinetic Properties

Monensin is rapidly absorbed following oral administration. Ruminants absorb only about 50% of a dose, while monogastric species appear to absorb almost all of an administered dose. Ionophores do not accumulate in large amounts in tissues, even when toxic doses are administered orally (Donoho, 1984). Ionophores are rapidly and extensively metabolized by the liver into numerous metabolites, which are excreted in the bile and eliminated in the feces. Horses are not able to eliminate monensin from the blood as rapidly as cattle, which may partly explain why horses are the species most sensitive to monensin toxicity.

Toxicity and Adverse Effects

The relative toxicities of the ionophores from lowest to highest are salinomycin < lasalocid \leq narasin < monensin < maduramicin (Oehme and Pickrell, 1999). Ionophore toxicity causes cellular electrolyte imbalances, elevating extracellular potassium and intracellular calcium, resulting in severe cellular damage and death. The toxic dose is variable among species, with equine species being the most sensitive and turkeys being more sensitive than chickens (Table 18.1). Skeletal and cardiac muscle cells are generally the most severely affected; however, the specific tissues affected and resulting clinical signs vary from species to species. Skeletal muscle is primarily affected in dogs, ostriches, sheep and turkeys. Cardiac muscles are affected in cattle, and both myocardium and skeletal

Table 18.1. Ionophore toxicity by drug and species.

Drug	Species	Toxicity
Lasalocid	Horses	LD ₅₀ is 15 mg/kg.
	Cattle	10-50 mg/kg causes depression, ataxia, paresis, inappetance, labored breathing, cardiomyopathy. 100-125 mg/kg is fatal.
Maduramicin Monensin	Chickens	LD ₅₀ is 71.5 mg/kg.
	Cattle	6 mg/kg of feed caused 50% mortality in calves.
	Cattle	20-40 mg/kg caused cardiotoxicity in calves.
	Chickens	LD ₅₀ is 200 mg/kg.
	Deer	225 mg/kg of feed caused cardiomyopathy and death.
	Dogs	LD ₅₀ is 20 mg/kg.
	Goats	15 mg/kg daily for 3 months caused ataxia, cardiomyopathy, depression, diarrhea, muscle weakness, paresis, weight loss.
		LD ₅₀ is 26 mg/kg.
	Horses	50 mg/kg daily for two weeks caused death.
		LD ₅₀ is 2-3 mg/kg.
		125 mg/kg of feed for 28 days caused toxicity.
		279 mg/kg of feed for 1-3 days caused death.
	Pigs	LD ₅₀ is 17 mg/kg.
	Ostriches	3-4 mg/kg daily for 13 days caused toxicity and death.
	Sheep	12 mg/kg.
	Turkeys	90 mg/kg of feed caused no adverse effects.
Naracin	Dogs	180-450 mg/kg of feed caused toxicity and death.
		LD ₅₀ is 3-10 mg/kg.
Salinomycin	Rabbits	2 mg/kg daily results in mild toxicity in adults but more severe toxicity in puppies.
		LD ₅₀ is 10.75 mg/kg.
	Cattle	90 mg/kg of feed for 4-7 weeks caused toxicity and death.
Semduramicin	Turkeys	13-18 mg/kg of feed caused toxicity and death.
	Chickens	50-75 mg/kg of feed reduced feed intake, rate of weight gain, and feather quality.

muscles are damaged in horses. Age-related differences in ionophore sensitivity occur in poultry, with adult birds more sensitive to the toxic effects of ionophores than young birds. In dogs, puppies are more sensitive to the toxic effects of narasin than adult dogs. In cattle, calves 5 to 8 months of age are much more susceptible to the toxic effects of maduramicin exposure than calves aged 9 to 16 months.

Ionophore toxicity occurs from dose errors in mixing with feed, accidental ingestion of treated feed by sensitive species, ingestion by ruminants of poultry litter from ionophore-treated flocks, concurrent administration with a medication that potentiates toxicosis, or accidental feed mill contamination of feed. Heat stress and water deprivation exacerbate toxicity in chickens when lasalocid is administered at one to two times the recommended dose. Cattle and sheep have manifested signs of ionophore toxicity following ingestion of poultry litter from chicken flocks treated with maduramicin (Bastianello et al., 1995). Toxicity might also occur if poultry litter from flocks treated

with other ionophore antibiotics is fed to ruminants. Ionophore toxicosis is potentiated by medications that interfere with hepatic metabolism. Tiamulin administered concurrently with monensin caused signs of severe ionophore toxicity in chickens and pigs (Szucs et al., 2004; Umemura et al., 1985).

Bibliography

- Bastianello SS, et al. 1995. Cardiomyopathy of ruminants induced by the litter of poultry fed on rations containing the ionophore antibiotic, maduramicin. II. Macropathology and histopathology. *Onderstepoort J Vet Res* 62:5.
- Bergen WG, Bates DB. 1984. Ionophores: their effect on production efficiency and mode of action. *J Anim Sci* 58:1465.
- Callaway TR, et al. 2003. Ionophores: their use as ruminant growth promotants and impact on food safety. *Curr Issues Intest Microbiol* 4:43.
- Donoho AL. 1984. Biochemical studies on the fate of monensin in animals and in the environment. *J Anim Sci* 58:1528.

- Kronfeld DS. 2002. Lasalocid toxicosis is inadequately quantified for horses. *Vet Hum Toxicol* 44:245.
- Oehme FW, Pickrell JA. 1999. An analysis of the chronic oral toxicity of polyether ionophore antibiotics in animals. *Vet Hum Toxicol* 41:251.
- Szucs G, et al. 2004. Biochemical background of toxic interaction between tiamulin and monensin. *Chem Biol Interact* 147:151.
- Todd GC, et al. 1984. Comparative toxicology of monensin sodium in laboratory animals. *J Anim Sci* 58:1512.
- Umemura T, et al. 1985. Enhanced myotoxicity and involvement of both type I and II fibers in monensin-tiamulin toxicosis in pigs. *Vet Pathol* 22:409.

Clinical Applications

Lasalocid

Lasalocid is approved in the United States for the control of coccidiosis in cattle, chickens, and sheep. In Canada, it is approved for control of coccidiosis in cattle and chickens. In both countries it is approved for growth promotion and feed efficiency in cattle. A total oral dose of 200 mg per animal per day initiated six days prior to tryptophan exposure is effective under experimental conditions for the prevention of acute bovine pulmonary edema and emphysema (fog fever) in cattle. Continuing lasalocid for ten days following abrupt change in pasture appears to protect cattle during the critical period. This dose is within the labeled dose range for other indications. There is some evidence to suggest that the dose labeled for growth promotion is effective in preventing grain bloat in cattle (Bartley et al., 1983).

Maduramicin

Maduramicin is approved as a premix for coccidiosis control in chickens in the United States and in chickens and turkeys in Canada. Reduced rate of growth and no improvement in feed efficiency occurs if fed at concentrations of 6 ppm to chickens not suffering from coccidiosis.

Monensin

Monensin is a fermentation product of *Streptomyces cinnamomensis*. It is active against Gram-negative bacteria, some *Campylobacter* spp., and *Brachyspira* (*Serpulina*) *hyodysenteriae* (MIC 0.1 µg/ml), as well as against coccidia and *Toxoplasma*. Its antimicrobial effect in the rumen influences the production of volatile fatty acids, which promotes growth and feed efficiency and helps prevent bloat in beef cattle and ketosis in dairy cattle (Gallardo et al., 2005). Monensin prevents

clinical signs of tryptophan-induced acute bovine pulmonary edema in cattle (Potchoiba et al., 1992) and appears to reduce the development of lactic acidosis after grain overload in cattle (Burrin and Britton, 1986). Monensin may reduce abortion and control neonatal losses from toxoplasmosis in sheep (Buxton et al., 1988.). Monensin supplementation decreased the duration of shedding in *E. coli* O157:H7 positive cows on a forage diet (Van Baale et al., 2004).

In the United States, monensin is available as a feed premix for use in beef cattle for improved feed efficiency and coccidiosis control, for improved milk production in lactating dairy cattle, and for coccidiosis control in bobwhite quail, chickens, turkeys, and goats. In Canada, the monensin premix is only approved for improved feed efficiency in beef cattle and coccidiosis control in chickens, turkeys, and calves. Monensin is available in Canada as controlled-release capsules to prevent legume bloat in beef cattle and subclinical ketosis in lactating dairy cattle. When administered, the monensin capsule's embossed number should be recorded with the corresponding animal identification number, and cattle should be observed for 1 hour following treatment. If the capsule is regurgitated, the animal is identified and re-treated with an undamaged capsule. Cattle treated with monensin capsules should be checked for 4 days following treatment for bloat, coughing, drooling, and inappetence, which could indicate that the capsule is lodged in the esophagus. Regurgitated capsules must be disposed of properly as they can be lethal to dogs if chewed.

Narasin and Semduramicin

In the United States and Canada, narasin and semduramicin are approved for coccidiosis control in chickens.

Salinomycin

Salinomycin is approved in the United States and Canada for coccidiosis control in chickens and in Canada for growth promotion and feed efficiency in cattle and swine. Salinomycin is toxic to turkeys and causes mortality at the label dose for chickens (Andreasen et al., 1995).

Bibliography

- Andreasen JR, et al. 1995. Salinomycin toxicosis in male breeder turkeys. *Avian Dis* 39:638.

- Bartley EE, et al. 1983. Effects of lasalocid or monensin on legume or grain (feedlot) bloat. *J Anim Sci* 56:1400.
- Burrin DG, Britton RA. 1986. Response to monensin in cattle during subacute acidosis. *J Anim Sci* 63:888.
- Buxton D, et al. 1988. Further studies in the use of monensin in the control of experimental ovine toxoplasmosis. *J Comp Pathol* 98:225.
- Gallardo MR, et al. 2005. Monensin for lactating dairy cows grazing mixed-alfalfa pasture and supplemented with partial mixed ration. *J Dairy Sci* 88:644.
- Nocerini MR, et al. 1985. Reduction of 3-methylindole production and prevention of acute bovine pulmonary edema and emphysema with lasalocid. *J Anim Sci* 60:232.
- Potchoiba MJ, et al. 1992. Effect of monensin and supplemental hay on ruminal 3-methylindole formation in adult cows after abrupt change to lush pasture. *Am J Vet Res* 53:129.
- Van Baale MJ, et al. 2004. Effect of forage or grain diets with or without monensin on ruminal persistence and fecal *Escherichia coli* O157:H7 in cattle. *Appl Environ Microbiol* 70:5336.

Nitrofurans

Nitrofurans are broad-spectrum antimicrobials, but their toxicity now limits their use to topical application. While they had some clinical use for the treatment of intestinal and urinary tract infections, their carcinogenicity has led to their ban for use in food animals in the United States, Canada, and the European Union. However some nitrofurans, such as nitrofurantoin and nifuroxazide, are still used as antibacterial agents in humans. Nitrofurantoin is used as first-line prophylaxis therapy for acute or recurrent urinary tract infections and nosocomial urinary tract infections, and nifuroxazide is used for therapy of acute bacterial diarrhea. However, like other nitrofuran derivatives, these two compounds are mutagenic in bacteria (Hofnung et al., 2002). Nitrofurazone, once used orally as a veterinary antimicrobial drug, causes mammary and ovarian tumors in animals. Nitrofurazone stimulates the proliferation of estrogen-dependent cells, and nitrofurazone metabolites are involved in tumor initiation through oxidative DNA damage. Nitrofurazone enhances cell proliferation, leading to promotion and/or progression in carcinogenesis (Hiraku et al., 2004).

The only approved products in the United States and Canada are topical formulations of nitrofurazone for use in non-food animals for skin infections. Intact nitrofurazone accumulates in whole eyes of chickens fed varying levels and may be used to monitor for abuse of this drug in poultry production (Cooper et al., 2005).

Bibliography

- Cooper KM, et al. 2005. Nitrofurazone accumulates in avian eyes—a replacement for semicarbazide as a marker of abuse. *Analyst* 130:824.
- Hiraku Y, et al. 2004. Mechanism of carcinogenesis induced by a veterinary antimicrobial drug, nitrofurazone, via oxidative DNA damage and cell proliferation. *Cancer Lett* 215(2):141.
- Hofnung M, et al. 2002. Genotoxicity of 2-nitro-7-methoxynaphtho[2,1-b]furan (R7000): a case study with some considerations on nitrofurantoin and nifuroxazide. *Res Microbiol* 153:427.

Nitroimidazoles

The nitroimidazoles include metronidazole, dimetridazole, ronidazole, tinidazole and ipronidazole. Like the nitrofurans, the nitroimidazoles were once widely used in veterinary medicine, but because of potential carcinogenicity, have now been banned for use in food animals in the United States, Canada and the European Union. Metronidazole is still used in companion animals for its excellent activity against anaerobes and protozoa.

Chemistry

Nitroimidazoles are heterocyclic compounds based on a five-membered nucleus similar to that of the nitrofurans (Figure 18.1).

Mechanism of Action

After entry into the cell, nitroimidazoles undergo reduction of the nitro group to produce a variety of unstable intermediates, including antibacterial products. Reduction occurs under anaerobic conditions, but unlike that of the nitrofurans, is not enzymatically controlled. The unstable intermediates interact with bacterial or protozoal DNA, damaging the helical structure and causing strand breakage. These effects inhibit nucleic acid synthesis and cause cell death. Nitroimidazoles also cause inhibition of the DNA repair enzyme DNAase I.

Antimicrobial Activity

The antimicrobial activities of the clinically useful nitroimidazoles are similar. They are bactericidal to most Gram-negative and many Gram-positive anaerobic bacteria (Table 18.2). They are highly active against *Brachyspira* (*Serpulina*) *hyodysenteriae* and a variety of

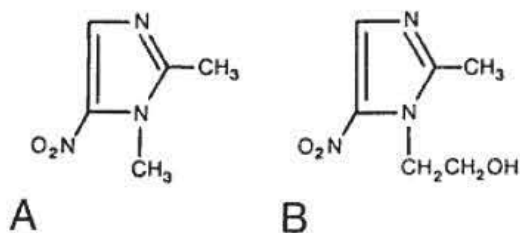


Figure 18.1. Structural formulas of nitroimidazole drugs: A, metronidazole; B, dimetridazole.

protozoa (*Tritrichomonas foetus*, *Giardia lamblia*, *Histomonas meleagridis*). *Campylobacter* spp. are moderately susceptible. *Helicobacter pylori* are commonly susceptible, but the susceptibility of animal-origin *Helicobacter* species has not been sufficiently investigated to substantiate clinical use (Happonen et al., 2000).

Resistance

Resistance is rare among usually susceptible bacteria. Resistance involves reduced intracellular drug activation. Cross-resistance between nitroimidazoles is complete. Equine and canine isolates of *Clostridium difficile* and *Clostridium perfringens* resistant to metronidazole have been occasionally described, so susceptibility testing is warranted in patients with clostridial diarrhea (Jang, et al., 1997a; Marks and Kather EJ, 2003). *Bacteroides fragilis* resistant to metronidazole therapy has been reported in a horse with pleuropneumonia (Dechant, 1997).

Pharmacokinetic Properties

Metronidazole is absorbed rapidly and well after oral administration in horses and dogs, with an oral bioavailability of 75–85% in horses and 59–100% in dogs (Neff-Davis et al., 1981; Steinman et al., 2000). In

horses with gastrointestinal ileus, metronidazole may be administered per rectum and is rapidly absorbed; however, the bioavailability is only 30%. Metronidazole is lipophilic and widely distributed in tissues. It penetrates bone, abscesses and the central nervous system. It crosses the placenta and is distributed into milk in concentrations similar to those in plasma. The volume of distribution is 0.7–1.7 L/kg in mares and 0.95 L/kg in dogs. Metronidazole is primarily metabolized in the liver by oxidation and conjugation. Both metabolites and unchanged drug are eliminated in urine and feces. The elimination half-life is 3–4 hours in horses and 8 hours in dogs.

Drug Interactions

No interference with the susceptibility of anaerobic bacteria has been reported in vitro when metronidazole is combined with a variety of other anaerobe-active drugs, such as clindamycin, erythromycin, penicillin G, amoxicillin-clavulanic acid, cefoxitin, and rifampin. Combined with a beta-lactam and gentamicin or enrofloxacin, metronidazole is commonly used for therapy of bacterial pleuropneumonia or peritonitis in horses (Mair and Yeo, 1987). The hepatic metabolism of metronidazole may be decreased when administered concurrently with cimetidine, possibly resulting in delayed elimination and increased serum concentrations of metronidazole. Phenobarbital induces microsomal liver enzymes; concurrent administration increases the metabolism of metronidazole and decreases serum concentrations.

Toxicity and Adverse Effects

Nitroimidazoles have been shown to be carcinogenic in some laboratory animals and mutagenic in some in vitro assays. These drugs are banned for use in food

Table 18.2. In vitro activity (MIC₉₀, µg/ml) of metronidazole against selected anaerobic bacteria.

Organism	MIC ₉₀	Organism	MIC ₉₀
Gram-positive anaerobes			
<i>Clostridium</i> spp.	4	<i>Actinomyces</i> spp.	>128
<i>C. perfringens</i>	2	<i>Eubacterium</i> spp.	4
<i>C. difficile</i>	0.5	<i>Peptostreptococcus</i> spp.	≤64
<i>C. septicum</i>	2	<i>Peptococcus</i> spp.	1
Gram-negative anaerobes			
All anaerobes	2	<i>Porphyromonas asaccharolytica</i>	2
<i>Bacteroides fragilis</i>	2	<i>Fusobacterium</i> spp.	0.5
<i>Bacteroides</i> spp.	2	<i>Brachyspira hyodysenteriae</i>	0.5

animals in the United States, Canada and the European Union, but metronidazole is still directly used in people, without reports of cancer-associated morbidity. Oral use in horses may occasionally cause anorexia, while vomiting, nausea and nervousness are seen in dogs and cats. Adverse effects of metronidazole in humans include seizures, ataxia, peripheral neuropathy, and hematuria. Adverse effects of metronidazole in the dog and cat include vomiting, hepatotoxicity, neutropenia, and neurologic signs such as seizures, head tilt, falling, paresis, ataxia, vertical nystagmus, tremors, and rigidity (Caylor and Cassimatis, 2001; Dow et al., 1989; Olson et al., 2005). Neurologic toxicity from metronidazole has been reported in dogs receiving 60 mg/kg/day for an average of 3–14 days, but there are reports of toxicity at lower dosages. The mechanism of the neurotoxic effects of metronidazole has not been identified. With discontinuation of the drug and supportive therapy, the reported recovery time of dogs with neurologic manifestations of metronidazole toxicosis is 1–2 weeks. The recovery time can be significantly shortened by the administration of diazepam, with an initial IV bolus of 0.5 mg/kg followed by oral administration of the same dose every 8 hours for 3 days (Evans et al., 2003). Recovery time is markedly shorter for diazepam-treated dogs (38.8 hours) compared to untreated dogs (11 days). While mechanism of this effect is unknown, it is likely that diazepam at therapeutic concentrations competitively reverses the binding of metronidazole to the benzodiazepine site on the GABA receptor.

Administration and Dosage

Since the antibacterial effect of nitroimidazoles is concentration-dependent, twice daily therapy is now recommended over three times daily therapy. All nitroimidazoles are now banned for use in food animals in the United States, Canada, and the European Union. There are no veterinary formulations of metronidazole, so human formulations are usually used. Metronidazole USP induces salivation and inappetence when administered orally to some cats. Products containing metronidazole benzoate are commercially available in some countries and the drug is available for formulation in the United States and Canada (Groman, 2000). Metronidazole benzoate is very well tolerated by cats. The recommended dose for treatment of giardiasis in dogs and cats is 25 mg/kg every 12 hours for 5–7 days. Lower doses (10–20 mg/kg

every 12 hours) are used chronically for the treatment of inflammatory bowel diseases. High doses (25–50 mg/kg every 12 hours) are sometimes used in the treatment of serious anaerobic infections (peritonitis, meningitis), but there is an increased risk of neurotoxicity. Doses of 15–25 mg/kg PO every 12 hours are used in horses; withholding feed for 2 hours after administration may improve bioavailability.

Clinical Applications

Metronidazole is used to treat anaerobic infections, especially pleuropneumonia and lung abscesses caused by penicillin-resistant *Bacteroides fragilis* and clostridial enterocolitis in horses (Baverud et al., 2003; Mair and Yeo, 1987). It is typically administered orally along with a parenteral beta-lactam and aminoglycoside or enrofloxacin to achieve Gram-positive, Gram-negative and anaerobic coverage. Although rectal absorption is inferior to oral absorption, it is a viable option for treatment when oral administration is not feasible.

In small animals, metronidazole is used to treat anaerobic infections, including bacterial stomatitis, osteomyelitis, hepatitis, pneumonia and lung abscessation, clostridial enteritis and peritonitis (Jang et al., 1997.b; Sarkiala and Harvey, 1993; Weese and Armstrong, 2003). It is also used in the treatment of giardiasis and other protozoal infections (*Trichomonas*, *Balantidium coli*). Metronidazole appears efficacious for the treatment of giardia in cats, but fenbendazole may be more effective in dogs, with fewer side effects (Barr et al., 1994; Scorza and Lappin, 2004). Metronidazole is sometimes effective in the treatment of inflammatory bowel diseases by inhibiting leukocyte-endothelial cell adhesion in postcapillary venules (Craven et al., 2004) and may be useful in the presurgical management of perianal fistulas (Tisdall et al., 1999). Oral administration of metronidazole decreased the number of aerobic bacteria and altered indigenous flora in the small bowel of cats (Johnston et al., 2000). The alteration in bacterial flora appeared to have an impact on nutrients, because serum albumin and cobalamin concentrations increased during administration and returned to pre-administration concentrations after therapy was discontinued.

Bibliography

- Barr SC, et al. 1994. Efficacy of fenbendazole against giardiasis in dogs. *Am J Vet Res* 55:988.

- Baverud V, et al. 2003. *Clostridium difficile*: prevalence in horses and environment, and antimicrobial susceptibility. *Equine Vet J* 35:465.
- Caylor KB, Cassimatis MK. 2001. Metronidazole neurotoxicosis in two cats. *J Am Anim Hosp Assoc* 37:258.
- Craven M, et al. 2004. Canine inflammatory bowel disease: retrospective analysis of diagnosis and outcome in 80 cases (1995–2002). *J Small Anim Pract* 45:336.
- Dechant J. 1997. Combination of medical and surgical therapy for pleuropneumonia in a horse. *Can Vet J* 38:499.
- Dow SW, et al. 1989. Central nervous system toxicosis associated with metronidazole treatment of dogs: five cases (1984–1987). *J Am Vet Med Assoc* 195(3):365.
- Evans J, et al. 2003. Diazepam as a treatment for metronidazole toxicosis in dogs: a retrospective study of 21 cases. *J Vet Intern Med* 17:304.
- Groman, R. 2000. Metronidazole. *Compendium on Continuing Education for the Practicing Veterinarian* 22, 1104.
- Happonen I, et al. 2000. Effect of triple therapy on eradication of canine gastric helicobacters and gastric disease. *J Small Anim Pract* 41:1.
- Jang SS, et al. 1997.a Antimicrobial susceptibilities of equine isolates of *Clostridium difficile* and molecular characterization of metronidazole-resistant strains. *Clin Infect Dis* 25 Suppl 2:S266.
- Jang SS, et al. 1997.b Organisms isolated from dogs and cats with anaerobic infections and susceptibility to selected antimicrobial agents. *J Am Vet Med Assoc* 210:1610.
- Johnston KL, et al. 2000. Effects of oral administration of metronidazole on small intestinal bacteria and nutrients of cats. *Am J Vet Res* 61:1106.
- Mair TS, Yeo SP. 1987. Equine pleuropneumonia: the importance of anaerobic bacteria and the potential value of metronidazole in treatment. *Vet Rec* 121:109.
- Marks SL, Kather EJ. 2003. Antimicrobial susceptibilities of canine *Clostridium difficile* and *Clostridium perfringens* isolates to commonly utilized antimicrobial drugs. *Vet Microbiol* 94:39.
- Neff-Davis CA, et al. 1981. Metronidazole: a method for its determination in biological fluids and its disposition kinetics in the dog. *J Vet Pharmacol Ther* 4:121.
- Olson EJ, et al. 2005. Putative metronidazole neurotoxicosis in a cat. *Vet Pathol* 42:665.
- Sarkiala E, Harvey C. 1993. Systemic antimicrobials in the treatment of periodontitis in dogs. *Semin Vet Med Surg (Small Anim)* 8:197.
- Scorza AV, Lappin MR. 2004. Metronidazole for the treatment of feline giardiasis. *J Feline Med Surg* 6:157.
- Steinman A, et al. 2000. Pharmacokinetics of metronidazole in horses after intravenous, rectal and oral administration. *J Vet Pharmacol Ther* 23:353.
- Tisdall PL, et al. 1999. Management of perianal fistulae in five dogs using azathioprine and metronidazole prior to surgery. *Aust Vet J* 77:374.
- Weese JS, Armstrong J. 2003. Outbreak of *Clostridium difficile*-associated disease in a small animal veterinary teaching hospital. *J Vet Intern Med* 17:813.

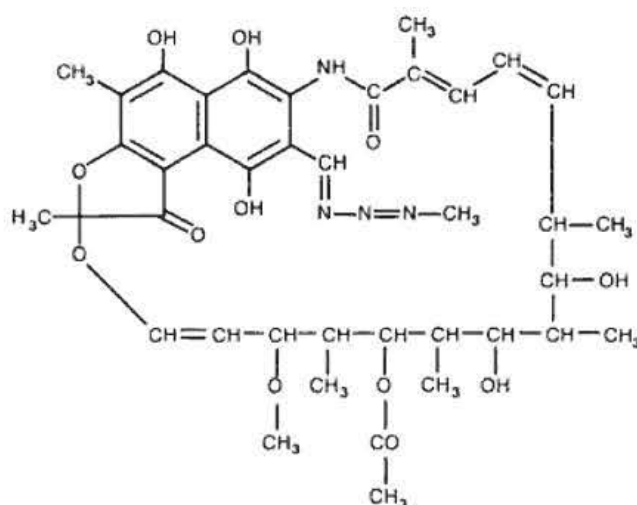


Figure 18.2. Structural formula of rifampin.

Rifamycins

Rifampin (Figure 18.2) is the most important synthetically modified member of the rifamycins, antibiotic products of *Ammycolaptopsis mediterranei*. Rifampin is a highly active first-line oral drug for the treatment of tuberculosis in humans. Because of the ready development of resistance, rifampin is always combined with other antimicrobials for therapy. Care must be taken, however, as rifampin is a potent hepatic enzyme inducer and there are numerous interactions with other drugs. In addition to antibacterial activity, rifampin also has some antiviral and antifungal activity. Rifabutin and rifapentine are other semisynthetic derivatives of rifamycin that are used in human medicine and have the advantage of causing less hepatic enzyme induction than rifampin.

Chemistry

Rifampin is an ansamycin, with an aromatic ring system spanned by an aliphatic bridge. It is soluble in organic solvents and in water at an acid pH.

Mechanism of Action

Rifampin inhibits DNA-dependent RNA polymerase in bacteria. At therapeutic doses, it does not affect mammalian RNA polymerase. Rifampin is bactericidal and active against both extracellular and intracellular pathogens. Rifampin enters neutrophils and macrophages to kill intracellular bacteria, without interfering with phagocytosis (Lobo and Mandell 1972).

Rifampin penetrates the outer membrane of Gram-positive bacteria more easily than that of Gram-negative bacteria (Frank, 1990).

Antimicrobial Activity

Rifampin is a broad-spectrum antibiotic, with activity against many Gram-positive and some Gram-negative aerobic bacteria as well as facultative anaerobic organisms. Rifampin is the most active antimicrobial drug against *Staphylococcus aureus*. Because of unpredictable susceptibilities, Gram-negative bacteria should be considered resistant unless indicated by susceptibility testing (Thornsberry et al., 1983). The ability of rifampin to reach intracellular bacteria makes it difficult to predict clinical results from in vitro susceptibility tests (Zak et al., 1985). Because infections often involve multiple pathogens and because of rapid development of resistance, rifampin is typically administered with other antimicrobial agents.

Rifampin is active against equine *Corynebacterium pseudotuberculosis*, *Rhodococcus equi*, *Staphylococcus* species, *Streptococcus equi*, *S. equisimilis*, and *S. zooepidemicus* isolates. Susceptibility is variable for the equine Gram-negative nonenteric bacteria. Rifampin has moderate activity against *Actinobacillus suis*, *A. equuli*, and *Pasteurella* spp. isolates. Equine isolates of

Pseudomonas aeruginosa, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus* spp., and *Salmonella* spp. are resistant (Wilson et al., 1988). Porcine isolates of *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* are susceptible to rifampin, but *Bordetella bronchiseptica* can be resistant. Human and animal strains of *Mycobacterium paratuberculosis* are susceptible (Chiodini, 1990). Anaerobes found to be susceptible in vitro include *Bacteroides fragilis* and *Fusobacterium* spp. (Bach and Thadepalli, 1980). Rifampin is one of the most active antimicrobials against *Chlamydia trachomatis* (MIC <0.25 $\mu\text{g/ml}$), where it kills in a dose-dependent manner and is synergistic with erythromycin (Siewert et al., 2005). Rifampin is effective against *Coxiella burnetii*, an obligate intracellular bacterium and causative agent of Q fever (Brennan and Samuel, 2003). Bacteria with MIC ≤ 2 $\mu\text{g/ml}$ are regarded as susceptible and those with MIC 2–4 $\mu\text{g/ml}$ as moderately susceptible (Table 18.3).

Resistance

Resistance to rifampin develops quickly by chromosomal mutation to high-level resistance. Resistant mutants may be concentration-sensitive and contain RNA polymerases with one of a variety of sensitivities to rifampin (Wehrli, 1983). Resistance may occur as a

Table 18.3. In vitro activity (MIC₉₀, $\mu\text{g/ml}$) of rifampin against selected bacteria.

Organism	MIC ₉₀	Organism	MIC ₉₀
Gram-positive aerobes			
<i>Bacillus anthracis</i>	0.03	<i>Staphylococcus aureus</i>	0.03
<i>Corynebacterium pseudotuberculosis</i>	≤ 0.25	<i>Rhodococcus equi</i>	0.06
<i>Enterococcus</i> spp.	≥ 4	<i>Nocardia</i> spp.	>256
<i>Listeria monocytogenes</i>	0.25	Beta-hemolytic streptococci	≤ 0.5
<i>Mycobacterium avium</i> complex	4		
<i>M. fortuitum</i>	>64		
<i>M. tuberculosis</i>	<0.03		
Gram-negative aerobes			
<i>Actinobacillus pleuropneumoniae</i>	0.5	<i>Escherichia coli</i>	16
<i>Bordetella bronchiseptica</i>	≥ 128	<i>Klebsiella pneumoniae</i>	32
<i>Brucella canis</i>	1	<i>Pasteurella</i> spp.	1
<i>B. abortus</i>	2	<i>Proteus</i> spp.	32
<i>Campylobacter jejuni</i>	>128	<i>Pseudomonas aeruginosa</i>	64
Gram-positive anaerobes			
<i>Actinomyces</i> spp.	0.06	<i>Clostridium</i> spp.	1
<i>Clostridium perfringens</i>	0.13	<i>C. septicum</i>	≤ 0.13
<i>C. difficile</i>	≤ 0.25	<i>Peptostreptococcus</i> spp.	32
Gram-negative anaerobes			
<i>Bacteroides fragilis</i>	1	<i>Porphyromonas asaccharolytica</i>	0.25
<i>Fusobacterium</i> spp.	2		

single-step mutation of the DNA-dependent RNA polymerase at a high rate (1 in 10^7 or 10^8 bacteria). Initial susceptibility can rapidly diminish as small populations of resistant cells soon outnumber susceptible cells. This effect can be moderated by combination antimicrobial treatment. One case of the development of resistant *Rhodococcus equi* in a foal treated with erythromycin and rifampin has been reported (Kenney et al., 1994). Cross-resistance among the different rifamycin derivatives occurs, and recently, cross-resistance to drugs unrelated to rifampin has been documented (Xu et al., 2005).

Pharmacokinetic Properties

Although parenteral pharmacokinetic studies have been performed in horses, rifampin is generally administered orally in animals (Burrows et al., 1985). Rifampin is rapidly absorbed after oral administration to people, calves, dogs, and horses, although bioavailability is low in horses (Frank, 1990; Wilson et al., 1988.). Oral dosing for horses is adjusted for poor bioavailability. Administration with food prolongs the time to maximum serum concentration in adult horses and people.

Rifampin is very lipophilic and penetrates most tissues including milk, bone, abscesses and the central nervous system. Rifampin is well-distributed into milk, with a milk to serum concentration ratio of 0.9–1.28 in sheep. Rifampin penetrates phagocytic cells to kill susceptible intracellular bacteria. Rifampin crosses the placenta and is teratogenic in rodents. Feces, saliva, sweat, tears, and urine are discoloured red-orange by rifampin and its metabolites. The volume of distribution of rifampin in horses is 0.6–0.9 L/kg. Rifampin is highly bound to plasma proteins in humans and horses. In horses, serum concentrations $>2 \mu\text{g/mL}$ are reached 45 minutes after intragastric administration of 20 mg/kg and serum concentrations are maintained at $>3 \mu\text{g/mL}$ for at least 24 hours. In dogs, serum concentrations are 9–10 $\mu\text{g/mL}$ 24 hours after a single oral dose of 10 mg/kg.

Rifampin causes induction of hepatic enzymes in many species. In humans, the primary metabolite of rifampin is 25-desacetylrifampin, which also has antibacterial activity. Human desacetylrifampin is more profusely secreted in bile than rifampin, but serum concentrations are lower than the parent drug (Frank, 1990). While rifampin undergoes extensive enterohepatic recycling in humans, desacetylrifampin is poorly absorbed from the intestine and not recycled. The bio-

transformation and elimination of rifampin in animals is not well known, and the major metabolites of the parent drug in most animals have not been determined. Desacetylrifampin was not detected in serum samples after IV or oral dosing in horses. It was detected in urine, but the parent compound was much more predominant; however, only 6.82% of the total dose was recovered in the urine as either compound. It is not known if the unrecovered rifampin is sequestered in tissues or excreted in bile as desacetylrifampin, a more polar and more easily excreted metabolite (Kohn et al., 1993).

The elimination half-life of rifampin in horses is 6–8 hours after IV administration and 12–13 after oral administration. Due to immature hepatic metabolism, elimination of rifampin is delayed in very young foals and the elimination half-life is 17.5 hours. In dogs, the elimination half-life is 8 hours. As a hepatic enzyme inducer, rifampin induces its own metabolism, so that multiple oral dosing significantly decreases the elimination half-life. Enzyme induction typically requires 5 days of therapy, but once initiated, may last for more than 2 weeks after discontinuation of treatment.

Drug Interactions

Microsomal enzyme induction from rifampin may shorten the elimination half-life and decrease plasma drug concentrations of chloramphenicol, corticosteroids, theophylline, trimethoprim, itraconazole, ketoconazole, warfarin, and barbiturates.

Rifampin has in vitro synergistic activity with erythromycin and trimethoprim and has an additive effect with ampicillin or penicillin G. Rifampin's activity in vitro can be antagonistic to gentamicin; but it is not certain how this interaction affects in vivo activity (Prescott and Nicholson, 1984).

Toxicity and Adverse Effects

There is little published information regarding the effects of rifampin in small animals; however, there is anecdotal information that a significant percentage of dogs receiving 5 to 10 mg/kg a day develop increases in hepatic enzymes that may lead to clinical hepatitis.

Administration and Dosage

Rifampin is available as human labelled capsules or suspension for oral administration or as a diluted solution for IV use. Most horses object to the taste of rifampin, so care must be taken to deposit a dose well

back on the tongue and rinse the horse's mouth afterwards. Oral dosing of rifampin in horses is adjusted for poor bioavailability. Use of oral doses for parenteral administration of rifampin could result in overdosage. Parenteral rifampin should be administered only by the intravenous route, not intramuscularly or subcutaneously.

Clinical Applications

The use of rifampin in food-producing animals is not approved in the United States or Canada; therefore, there are no tolerances or established withdrawal times and the global FARAD centers have no data from which to make withdrawal recommendations. The issue of whether rifampin should be used in food animals is further complicated by its link to hepatic tumors in one strain of mice. The significance of this link is not known, but any residue of a known carcinogen in animal products for human consumption is a violation of the Food, Drug, and Cosmetic Act of the United States. The United States Pharmacopoeia Veterinary Medicine Advisory Panel has concluded that rifampin should not be administered to food-producing animals.

Rifampin is primarily used in foals for the treatment of *Rhodococcus equi* at a dose of 5 mg/kg PO every 12 hours. Originally, it was combined with erythromycin, but because of adverse side effects from the erythromycin, combinations with new human-labelled macrolides have been investigated. The combination of clarithromycin and rifampin appears superior to erythromycin/rifampin or azithromycin/rifampin (Giguère et al., 2004). The combination of erythromycin and rifampin is effective for treatment of Potomac Horse Fever caused by *Neorickettsia risticii*. Rifampin is usually dosed in adult horses at 10 mg/kg PO every 12 hours.

Because of hepatotoxicity, rifampin is rarely used in dogs, and a safe dose has not been determined. It is occasionally used for the treatment of refractory staphylococcal pyoderma and atypical mycobacterial infections in combination with clarithromycin. Rifampin was part of successful antimicrobial therapy of *Brucella canis*-induced ocular inflammation in a dog (Vinayak et al., 2004).

Bibliography

Bach VT, Thadepalli H. 1980. Susceptibility of anaerobic bacteria in vitro to 23 antimicrobial agents. *Chemotherapy* 26:344.

- Brennan RE, Samuel JE. 2003. Evaluation of *Coxiella burnetii* antibiotic susceptibilities by real-time PCR assay. *J Clin Microbiol* 41:1869-1874.
- Burrows GE, et al. 1985. Rifampin in the horse: comparison of intravenous, intramuscular, and oral administrations. *Am J Vet Res* 46:442.
- Chiodini RJ. 1990. Bactericidal activities of various antimicrobial agents against human and animal isolates of *Mycobacterium paratuberculosis*. *Antimicrob Agents Chemother* 34:366.
- Frank LA. 1990. Clinical pharmacology of rifampin. *J Am Vet Med Assoc* 197:114.
- Giguère S, et al. 2004. Retrospective comparison of azithromycin, clarithromycin, and erythromycin for the treatment of foals with *Rhodococcus equi* pneumonia. *J Vet Intern Med* 18:568.
- Kenney DG, et al. 1994. Development of reactive arthritis and resistance to erythromycin and rifampin in a foal during treatment for *Rhodococcus equi* pneumonia. *Equine Vet J* 26:246.
- Kohn CW, et al. 1993. Pharmacokinetics of single intravenous and single and multiple dose oral administration of rifampin in mares. *J Vet Pharmacol Ther* 16:119.
- Lobo MC, Mandell GL. 1972. Treatment of experimental staphylococcal infection with rifampin. *Antimicrob Agents Chemother* 2: 195.
- Prescott JE, Nicholson VM. 1984. The effects of combinations of selected antibiotics on the growth of *Corynebacterium equi*. *J Vet Pharmacol Ther* 7:61.
- Siewert K, et al. 2005. Growth cycle-dependent pharmacodynamics of antichlamydial drugs. *Antimicrob Agents Chemother* 49:1852.
- Thornsberry C, et al. 1983. Rifampin: spectrum of antibacterial activity. *Rev Infect Dis* 5 Suppl 3:S412.
- Wehrli W. 1983. Rifampin: mechanisms of action and resistance. *Rev Infect Dis* 5 Suppl 3:S407.
- Wilson WD, et al. 1988. Pharmacokinetics, bioavailability, and in vitro antibacterial activity of rifampin in the horse. *Am J Vet Res* 49:2041.
- Vinayak A, et al. 2004. Clinical resolution of *Brucella canis*-induced ocular inflammation in a dog. *J Am Vet Med Assoc* 224:1804-1807, 1788.
- Xu M, et al. 2005. Cross-resistance of *Escherichia coli* RNA polymerases conferring rifampin resistance to different antibiotics. *J Bacteriol* 187:2783.
- Zak O, et al. 1985. Correlation of antibacterial activities of antibiotics in vitro and in animal models of infection. *J Antimicrob Chemother* 15 Suppl A:273.

Oxazolidinones

The oxazolidinones are a novel chemical class of synthetic antibacterial agents that target protein synthesis. The antibacterial activity of this class of agent was first described in 1987. Oxazolidinones were abandoned for some time after these earlier studies because of

bone marrow toxicity. In 2000, linezolid became the first oxazolidinone approved for clinical use in people. Many analogs are currently under development.

Mechanism of Action

Oxazolidinones reversibly block protein synthesis by binding to the 23s ribosomal RNA (rRNA) of the 50s ribosomal subunit, near the interface formed with the 30s ribosomal subunit. Linezolid binds near the chloramphenicol and lincomycin binding sites and competes with these agents for binding. Although they share binding sites, their mechanism of action is different, with chloramphenicol inhibiting peptide bond formation and linezolid inhibiting initiation complex formation. As a result, there is only rare cross-resistance between linezolid and chloramphenicol or lincomycin (Hancock, 2004).

Antimicrobial Activity

In vitro, linezolid is active against many Gram-positive bacteria. It is bacteriostatic against staphylococci and enterococci and often bactericidal against streptococci. *Staphylococcus* spp. with a MIC ≤ 4 $\mu\text{g/ml}$, as well as *Enterococcus* spp. and *Streptococcus* spp. with a MIC ≤ 2 $\mu\text{g/ml}$ are considered susceptible to linezolid. Isolates with a MIC ≥ 8 $\mu\text{g/ml}$ are considered resistant. Linezolid is active against staphylococci including methicillin-resistant *S. aureus* and *S. epidermidis*. It is also active against *S. aureus* isolates with intermediate susceptibility to vancomycin. Linezolid is active against *Enterococcus faecium* and *E. faecalis*, including isolates resistant to vancomycin, and against *Listeria monocytogenes* and *Rhodococcus equi*. Linezolid does not have clinically useful activity against aerobic Gram-negative bacteria. It is active against most anaerobes including *Clostridium perfringens*, *C. difficile*, *Peptostreptococcus* spp., and *Fusobacterium* spp. *Bacteroides fragilis* isolates are resistant or have intermediate susceptibility.

Resistance

Linezolid retains activity against Gram-positive cocci resistant to other antibacterial agents. In addition, it is difficult to induce in vitro resistance to linezolid because it has a very low spontaneous resistance mutation rate. However, rare Gram-positive clinical isolates with mutations in their 23s rRNA conferring resistance to linezolid have been described (Herrero et al., 2002).

Pharmacokinetics

Linezolid is available in oral and parenteral forms. Rapid and extensive absorption occurs after oral administration in people and dogs with a bioavailability greater than 95% and maximum serum concentrations achieved less than 2 hours following administration (Slatter JG et al., 2002). The half-life of linezolid in dogs is approximately 4 h (Slatter JG et al., 2002). The drug is well distributed in all body tissues including the CSF, with only 30% of the drug being protein bound.

Toxicity and Adverse Effects

Clinical trial data in people indicate minimal adverse effects from linezolid. Most commonly reported adverse reactions in clinical trials involving more than 2000 patients included diarrhea (8.3%), headache (6.5%), nausea (6.2%), and vomiting (3.7%). Myelosuppression has been reported in patients receiving linezolid. Complete blood counts should be monitored weekly, particularly in patients who receive linezolid for longer than two weeks, those with pre-existing myelosuppression, and those receiving concomitant drugs that produce bone marrow suppression. The safety of linezolid at clinically relevant dosages has not been established in domestic animal species.

Clinical Indications

Linezolid has been marketed in people for the treatment of infection due to vancomycin-resistant *E. faecium*, nosocomial and community-acquired pneumonia due to *S. aureus* or multi-drug resistant *Streptococcus pneumoniae*, and skin infections including those caused by methicillin-resistant *Staphylococcus* spp. (Schmidt-loanas et al., 2005). There are no published reports of the clinical use of linezolid in domestic animals. In companion animals, linezolid could be useful for the treatment of infection caused by Gram-positive cocci resistant to other more commonly used antimicrobial agents. The decision to use linezolid to treat a highly resistant pathogen in a veterinary patient should only be made after consideration of the health risks to in-contact humans and other animals.

Bibliography

Hancock RE. 2004. Mechanisms of action of newer antibiotics for Gram-positive pathogens. *Lancet Infect Dis* 5:209.

- Herrero IA, et al. 2002. Nosocomial spread of linezolid-resistant, vancomycin-resistant *Enterococcus faecium*. *N Engl J Med* 346:867.
- Slatter JG, et al. 2002. Pharmacokinetics, toxicokinetics, distribution, metabolism and excretion of linezolid in mouse, rat and dog. *Xenobiotica*. 2002 32:907.
- Schmidt-Ioannas M., et al. New antibiotics for the treatment of severe staphylococcal infection in the critically ill patient. *Curr Opin Crit Care* 11:481.

Arsenicals

Arsenic-containing compounds (arsanilic acid, sodium arsanilate, 3-nitro-4-hydroxyphenylarsonic acid [roxarsone]) are used for growth promotion in swine and poultry. The toxicity of arsenic varies with several factors: its chemical form, oxidation states, and solubility. The phenylarsonic compounds are the least toxic and are used as feed additives in swine and poultry rations. However, roxarsone has a higher absolute toxicity than arsanilic acid. The mechanism of action is related to its reaction with sulfhydryl groups necessary for enzyme function and to its ability to uncouple oxidative phosphorylation (el Bahri and Ben Romdane, 1991; Rice et al., 1985). Symptoms of toxicity in swine include muscle tremors and clonic convulsive episodes, which progress to paraparesis and paraplegia (Blakley et al., 1990; Rice et al., 1985). Most animals excrete arsenic quite readily. Sodium arsanilate causes carrier swine to develop swine dysentery diarrhea and may have potential in identifying the carrier state.

Bibliography

- Blakley BR, et al. 1990. Roxarsone (3-nitro-4-hydroxyphenylarsonic acid) poisoning in pigs. *Can Vet J* 31:385.
- el Bahri L, Ben Romdane S. 1991. Arsenic poisoning in livestock. *Vet Hum Toxicol* 33:259.
- Olson LD, Rodabaugh DE. 1986. Feeding sodium arsanilate for exciting diarrhea and identifying carriers of swine dysentery. *Can J Vet Res* 50:359.
- Rice DA, et al. 1985. Experimental 3-nitro-4-hydroxyphenylarsonic acid toxicosis in pigs. *Res Vet Sci* 39:47.

Carbadox

Carbadox is a quinoxaline NN dioxide derivative used to promote growth and for prevention and control of dysentery and bacterial enteritis in pigs. In many areas

of the world it is used in animals up to 4 months of age with a 4-week withdrawal period prior to slaughter for human consumption. Other quinoxalines used as growth promoters in animals in some countries include olaquinox and cyadox. Carbadox inhibits bacterial DNA synthesis and denatures preexisting DNA. It is more active under anaerobic than aerobic conditions and its effect on DNA, like that of the nitrofurans, is believed to be caused by an unstable quinoxin-reduction product.

Carbadox is highly active against clostridia (MIC ≤ 0.25 $\mu\text{g/ml}$), *Brachyspira* (*Serpulina*) *hyodysenteriae* (MIC < 0.005 $\mu\text{g/ml}$), and aerobic bacteria under anaerobic conditions. Quinoxalines have some activity against *Chlamydia*/*Chlamydophila* spp. and protozoa. Resistance in field cases of swine dysentery has been described, but the mechanism has not been elucidated.

Carbadox is used for growth promotion and feed efficiency in swine at 10–25 ppm in feed. Concentrations of 50–55 ppm are used to prevent and treat swine dysentery and proliferative intestinal adenomatosis.

Doses of carbadox as low as 50 ppm induces hypoadosteronism from dose- and time-dependent damage to the zona glomerulosa of the adrenal cortex (Van der Molen, 1988). Also at 50 ppm, mild effects of increased fecal dryness, urine drinking, growth retardation and poor condition are seen. At 300 ppm, posterior paresis and death may occur (Power et al., 1989). Olaquinox causes similar toxicity in pigs, but cyadox is less toxic (Nabuurs et al., 1990).

Carbadox remains approved for use in swine in the United States, but is banned in Canada, Australia and the European Union because of carcinogenicity concerns. In 2003, Health Canada requested that the Joint Expert Committee on Food Additives (JECFA) review the safety of carbadox residues and the analytical methodology used to assess these products. Carbadox and some of its metabolites (desoxycarbadox and hydrazine) were found to be genotoxic and carcinogenic in rodents. The final metabolite, quinoxaline-2-carboxylic acid (QCA), was not found to be carcinogenic or mutagenic in animals. Initial studies of residues showed rapid depletion of carbadox and its genotoxic metabolites in liver and muscle to concentrations of < 2 $\mu\text{g/kg}$, within the limit of detection of the analytical method available at that time (MacIntosh et al., 1985). QCA was the most persistent metabolite and was the only residue detectable in edible tissues of pigs 72 hours after dosing. After a 28-day

withdrawal period, its concentration was $<30 \mu\text{g/kg}$ in liver and $5 \mu\text{g/kg}$ in muscle, representing the limits of quantification of the analytical method used at that time. Carbadox was reviewed by JECFA primarily on the basis of new information on residue concentrations, which indicated that the metabolite desoxycarbadox was present in edible tissues even at the end of a 15-day experimental withdrawal period. Reports of misuse and cross-contamination of swine finishing rations, combined with a better analytical capacity to detect desoxycarbadox, raised human safety concerns over the use of carbadox. (Vilim and Lambert, 2001). The Committee confirmed that both carbadox and desoxycarbadox should be regarded as carcinogens that act by a genotoxic mechanism. The Committee concluded that it was not possible to identify a dose of carbadox in swine that posed an acceptable risk to consumers. The Committee therefore did not establish an acceptable daily intake (ADI) for carbadox.

Olaquinox has been withdrawn from use in Europe because of cases of a photoallergic "phototoxic contact dermatitis" that developed in pig farmers.

Bibliography

- MacIntosh A, et al. 1985. Liquid chromatographic monitoring of the depletion of carbadox and its metabolite desoxycarbadox in swine tissues. *J Assoc Off Anal Chem* 68:665.
- Nabuurs MJ, et al. 1990. Clinical signs and performance of pigs treated with different doses of carbadox, cyadox and olaquinox. *Zentralbl Veterinarmed A* 37:68.
- Power SB, et al. 1989. Accidental carbadox overdosage in pigs in an Irish weaner-producing herd. *Vet Rec* 124:367.
- van der Molen EJ. 1988. Pathological effects of carbadox in pigs with special emphasis on the adrenal. *J Comp Pathol* 98:55.
- Vilim A, Lambert, G. 2001. Health risk assessment: Carbadox in swine. Human Safety Division, Bureau of Veterinary Drugs, Health Products and Food Branch, Ottawa: Health Canada.

Fusidic Acid

Fusidic acid is a lipophilic steroid antibiotic, a fusidane (like cephalosporin P_1 and helvolic acid). It is a product of *Fusidium coccineum* and available as a readily soluble sodium salt. It prevents protein synthesis by inhibiting the binding of aminoacyl tRNA to the ribosomal A site. Sodium fusidate is active mainly against

Gram-positive bacteria; it has excellent bactericidal activity against *Staphylococcus aureus* (MIC $\leq 0.03 \mu\text{g/ml}$) and moderate activity against *Mycobacterium tuberculosis* (MIC 0.5–2 $\mu\text{g/ml}$). Gram-negative rods are resistant. Resistant strains of *S. aureus* emerge readily in vitro and sometimes during treatment. Combination with penicillin prevents the emergence of resistant mutants. Fusidic acid is used orally in humans for the treatment of serious staphylococcal infections. It has been used topically in dogs for local treatment of staphylococcal infections (Cobb et al., 2005; Saijonna-Koulumies et al., 1998), but resistance by *Staphylococcus intermedius* has been documented (Guardabassi et al., 2004). It is available in some countries as an ophthalmic ointment for the treatment of Gram-positive bacterial keratitis in dogs and cats.

Bibliography

- Cobb MA, et al. 2005. Topical fusidic acid/betamethasone-containing gel compared to systemic therapy in the treatment of canine acute moist dermatitis. *Vet J* 169:276.
- Guardabassi L, et al. 2004. Transmission of multiple antimicrobial-resistant *Staphylococcus intermedius* between dogs affected by deep pyoderma and their owners. *Vet Microbiol* 98(1):23.
- Saijonna-Koulumies L, et al. 1998. Elimination of *Staphylococcus intermedius* in healthy dogs by topical treatment with fusidic acid. *J Small Anim Pract* 39:341.

Isoniazid

Isoniazid is the hydrazide of isonicotinic acid, a low-molecular weight, water-soluble drug. It is the most potent antituberculosis drug used in humans, and is bactericidal to *Mycobacterium tuberculosis* at concentrations of 0.05–0.2 $\mu\text{g/ml}$. *M. bovis* is similarly susceptible, but *M. avium-intracellulare* and other atypical mycobacteria are resistant. Many *M. kansasii* are susceptible. Among other genera, *Rhodococcus equi*, *Corynebacterium pseudotuberculosis*, and *Streptococcus zooepidemicus* are resistant. *Actinomyces bovis* is susceptible. Isoniazid is always administered in combination with other antimicrobials, because bacteria readily develop resistance (about 1 in 10^6). The antibacterial mechanism of action of isoniazid is unknown.

Isoniazid is well absorbed from the intestine and distributes well into tissues, including cerebrospinal fluid. Toxic effects occur in people who are genetically

slow acetylators of isoniazid (Kinzig-Schippers et al., 2005). Isoniazid has been used in cattle for the treatment of actinomycosis (Watts et al., 1973). It was ineffective in the treatment of Johne's disease in cattle. Isoniazid is not approved for use in food animals and there is no information available on pharmacokinetics or residue depletion.

A single dose of 300 mg isoniazid in dogs causes life-threatening central nervous system toxicity. As with metronidazole, diazepam is antidotal by improving GABAergic transmission in the central nervous system, and has proved effective in protecting animals from further convulsions and death (Doherty, 1982; Villar et al., 1995).

Bibliography

- Doherty T. 1982. Isoniazid poisoning in a dog. *Vet Rec* 111:460.
 Kinzig-Schippers M, et al. 2005. Should we use N-acetyltransferase type 2 genotyping to personalize isoniazid doses? *Antimicrob Agents Chemother* 49:1733.
 Villar D, et al. 1995. Treatment of acute isoniazid overdose in dogs. *Vet Hum Toxicol* 37:473.
 Watts TC, et al. 1973. Treatment of bovine actinomycosis with isoniazid. *Can Vet J* 14:223.

Mupirocin

Mupirocin (pseudomonic acid) is a novel antibiotic, isolated from *Pseudomonas fluorescens*. By preventing the incorporation of isoleucine into protein chains, this powerful inhibitor of bacterial isoleucyl transfer RN synthetase (IleS) stops protein synthesis. It has excellent activity in vitro against staphylococci and streptococci, but less activity against other Gram-positive and Gram-negative bacteria. Its activity is slowly bactericidal. Activity against multiple antibiotic-resistant *Staphylococcus aureus* is slightly less than its excellent activity against most skin staphylococcal isolates (<0.12 µg/ml). Mupirocin is bacteriostatic but appears to be bactericidal at a lower pH approximating that of many parts of the skin. It is rapidly metabolized after systemic administration, so it is only used topically.

Mupirocin was introduced into clinical practice in the United Kingdom in 1985, and has proved to be an extremely effective treatment of skin infections and

one of the most successful topical antibiotics for the clearance of nasal *Staphylococcus aureus* isolates including those resistant to methicillin. The skin ointment (with polyethylene glycol) and nasal cream (with soft paraffin) are currently registered for use in more than 90 countries worldwide. Bacterial resistance soon emerged with clinical use. Low-level resistance is probably due to mutations in a chromosomally encoded IleS, is stable and non-transferable. High-level resistance has been shown to be due in vivo to the acquisition of an additional novel IleS. Cross resistance with other antimicrobials does not occur, due to mupirocin's novel mechanism of action (Cookson, 1998). A dog served as a reservoir for mupirocin and methicillin-resistant *Staphylococcus aureus* (MRSA) colonization in its owners. The MRSA infection and nasal colonization in the couple was prevented only after successful eradication of MRSA from the family dog's nares with a vancomycin ointment (Manian, 2003).

Mupirocin is available as a veterinary product in the United States for pyoderma in dogs; however, in Canada, it is only available as the human labelled product. Owners can decrease the relapse rate and severity of pyoderma if they immediately apply mupirocin every 12 hours when they first notice early lesions developing. It is well tolerated in cats for the treatment of feline acne (White et al., 1997.). The mupirocin ointment penetrates well into granulomatous lesions such as interdigital abscesses.

Bibliography

- Cookson BD. 1998. The emergence of mupirocin resistance: a challenge to infection control and antibiotic prescribing practice. *J Antimicrob Chemother* 41(1):11.
 Manian FA. 2003. Asymptomatic nasal carriage of mupirocin-resistant, methicillin-resistant *Staphylococcus aureus* (MRSA) in a pet dog associated with MRSA infection in household contacts. *Clin Infect Dis* 36:e26.
 White SD, et al. 1997. Feline acne and results of treatment with mupirocin in an open clinical trial: 25 cases (1994–96). *Vet Dermatol* 8:157.

Methenamine

Methenamine (hexamine) is a highly soluble, basic substance of the chemical formula (CH₂)₆N₄, which decomposes under acidic conditions to release formaldehyde. It is available as a salt of mandelic acid or hip-

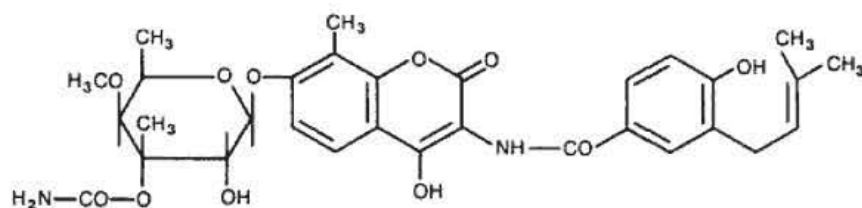


Figure 18.3. Structural formula of novobiocin.

puric acid. After oral administration of enteric-coated tablets, methenamine is well absorbed and excreted unchanged in urine. If the urine is strongly acidic (pH <5.5), methenamine releases formaldehyde, which acts as a nonspecific urinary antiseptic. It is usual to ensure urine acidity by concurrent administration of ascorbic acid or ammonium chloride. The drug is used for long-term prophylaxis of recurrent urinary tract infections in dogs and cats at 0.25 mg/15 kg every 6 hours. Urease-producing bacteria such as staphylococci and *Proteus* that make urine strongly alkaline through the release of ammonia from urea are usually not susceptible to methenamine.

Novobiocin

Novobiocin (Figure 18.3) is an antibiotic product of *Streptomyces* that is occasionally used in the local treatment of *Staphylococcus aureus* infections, including mastitis in dairy cows. Novobiocin is a coumarin antibiotic, formulated as a dibasic acid available as a

poorly water soluble calcium salt or as the more soluble monosodium salt.

Novobiocin inactivates the beta subunit of DNA gyrase, inhibiting supercoiling, DNA-dependent adenosine triphosphatase, and catenation/uncatenation.

Novobiocin is very active against *S. aureus*, less active against streptococci and the more fastidious Gram-negative bacteria (*Histophilus*, *Brucella*), and least active against Enterobacteriaceae and *Pseudomonas* (Table 18.4). In a study of bovine mastitis isolates, 95% of *S. aureus*, 60% of *Streptococcus dysgalactiae*, and 40% of *S. agalactiae* were susceptible to the drug. Many mycoplasma are moderately susceptible.

Novobiocin is usually bacteriostatic; activity is decreased by alkaline conditions and the presence of magnesium. Bacteria with MIC ≤ 4 $\mu\text{g/ml}$ are regarded as susceptible, MIC 8 $\mu\text{g/ml}$ as intermediate, and MIC ≥ 16 $\mu\text{g/ml}$ as resistant. Chromosomal resistance develops fairly readily in vitro and has been reported during treatment of *S. aureus* infection.

Moderate synergism with penicillin G against bovine *S. aureus* and streptococci has been described.

Table 18.4. In vitro activity (MIC₉₀, $\mu\text{g/ml}$) of novobiocin against selected bacteria.

Organism	MIC ₉₀	Organism	MIC ₉₀
Gram-positive aerobes			
<i>Arcanobacterium pyogenes</i>	64	<i>Rhodococcus equi</i>	>64
<i>Corynebacterium bovis</i>	1	<i>Staphylococcus aureus</i>	2*
<i>Enterococcus faecalis</i>	64	<i>Streptococcus agalactiae</i>	16
<i>Erysipelothrix rhusiopathiae</i>	>64	<i>S. pyogenes</i>	4
<i>Listeria monocytogenes</i>	2	<i>S. uberis</i>	2*
Gram-positive anaerobes			
<i>Actinomyces</i> spp.	16	<i>Clostridium perfringens</i>	1
Gram-negative aerobes			
<i>Brucella canis</i>	2	<i>Pasteurella multocida</i>	16
<i>Escherichia coli</i>	>64	<i>Proteus</i> spp.	64
<i>Histophilus somni</i>	≤ 0.13	<i>Pseudomonas aeruginosa</i>	>64
<i>Mannheimia haemolytica</i>	64	<i>Taylorella equigenitalis</i>	2
Mycoplasma			
<i>M. ovipneumoniae</i>	8		

*Some reports are far higher because of resistance.

The claim that novobiocin is synergistic with tetracycline may be a laboratory artifact associated with magnesium chelation by tetracycline.

Novobiocin is well absorbed from the gastrointestinal tract in humans and has an elimination half-life of 2–4 hours. Penetration into tissues is relatively modest. The drug is mainly excreted in the bile, and enterohepatic recirculation occurs. Skin eruptions in humans are common. The drug has a profound depressant effect on hepatic metabolism. Eosinophilia, thrombocytopenia, and leukopenia are occasional side effects. Skin rashes may occur in cows treated with intramammary infusions containing novobiocin.

The main use of novobiocin in veterinary medicine is in the local treatment of *S. aureus* infections.

The drug is combined with procaine penicillin G in dry cow therapy, with reasonable clinical efficacy. Prepartum therapy of heifer mammary glands with penicillin-novobiocin significantly reduced the percentage of heifers and quarters infected with mastitis pathogens during early lactation.

Bibliography

- Oliver SP, et al. 2004. Influence of prepartum pirlimycin hydrochloride or penicillin-novobiocin therapy on mastitis in heifers during early lactation. *J Dairy Sci* 87:1727.
- Owens WE, et al. 2001. Prevalence of mastitis in dairy heifers and effectiveness of antibiotic therapy. *J Dairy Sci* 84:814.

Antifungal Chemotherapy

Steeve Giguère

In human medicine, there has been an increase in the number of documented fungal infections over the last two decades. The advances in patient management technologies and therapies such as bone marrow and solid organ transplant, new and more effective antibacterial chemotherapeutic agents, more aggressive use of antibacterial chemotherapy, and the rise in the numbers of patients with HIV infection are all factors contributing to this increase in infections caused by a variety of fungi. Some of these factors are also likely contributors to the increase in the incidence of fungal infections in domestic animals, including the increase in nosocomial infections that is causing considerable morbidity and mortality in hospitalized patients. In addition to immunosuppression, risk factors common in many hospitalized veterinary patients include malnutrition, indwelling catheters, and destruction of the host's normal microbial flora by potent broad-spectrum antibacterial drugs.

Until relatively recently, the range of antifungal drugs available for systemic use was limited to a few agents. The most effective of these, amphotericin B, is also highly toxic. As fungal infections became an important public health issue, newer agents have been developed that exhibit a broader spectrum of activity, attack different targets and have fewer side effects. However, despite these developments, the number of antifungal agents for systemic use remains limited (Table 19.1). This is because mammalian cells and fungal pathogens have many common cellular characteristics and there are very few drugs that target sites that are unique and important to the fungus without being toxic to humans or animals. The main sites of action of antifungal drugs are the: (i) cytoplasmic membrane (e.g., polyenes, azoles); (ii) cell wall (e.g., echinocandins, nikkomycins); and (iii) inhibitors of

DNA and protein synthesis (e.g., flucytosine, sorbarins) (Figure 19.1).

Antifungal Susceptibility Testing

In vitro antifungal susceptibility tests differ from the susceptibility tests performed against bacteria in that fungi may be in the form of a yeast or filamentous fungi. The Clinical and Laboratory Standards Institute (CLSI) has described standardized testing methods for both of these forms of fungi, M27 and M38, respectively. The M27 document is intended for the testing of yeast that cause invasive infections and include organisms such as *Candida* spp. and *Cryptococcus neoformans*. The M37 document describes testing methods for common filamentous fungi that cause invasive infections such as *Aspergillus*, *Fusarium*, *Rhizopus*, *Pseudallescheria* and the mycelial form of *Sporothrix schenckii*. The methods described in these CLSI documents have not been standardized for testing the yeast forms of the dimorphic fungi such as *Blastomyces*, *Coccidioides* and *Histoplasma*. For specific details on how to perform antifungal susceptibility tests the reader is referred to these documents.

Since antifungal susceptibility testing is not routinely performed in veterinary clinical microbiology laboratories, referral of isolates to laboratories that specialize in antifungal testing is recommended in most instances. However, this can result in a considerable increase in costs and an additional delay in obtaining the results. To compensate for this, the clinician should be familiar with the types of pathogenic fungi most likely to be encountered and the susceptibility of those fungi to the antifungal agents they have at their disposal. Such knowledge will facilitate the initiation of the appropriate empirical therapy. One should keep in mind, however, that the susceptibility

Table 19.1. Systemic and topical antifungal agents in use.

Class	Agent	Formulations ^a	Spectrum
Allylamine	Terbinafine	O, T	Broad-spectrum ^b
	Naftidine	T	Similar
Pyrimidine synthesis inhibitors	Flucytosine	O	Yeasts ^c , some <i>Aspergillus</i>
Azole (Imidazole)	Ketoconazole	O, T	Dermatophytes, yeasts, dimorphic fungi ^d
	Miconazole	T	Broad-spectrum
	Enilconazole	T	Broad-spectrum
	Clotrimazole	T	Broad-spectrum
	Others ^e	T	
Azole (Triazole)	Fluconazole	O, IV, T	Yeasts, dimorphic fungi
	Itraconazole	O, IV	Broad-spectrum
	Voriconazole	O, IV	Broad-spectrum
Echinocandin	Caspofungin	IV	<i>Candida</i> spp., <i>Aspergillus</i>
Polyene	Amphotericin B	IV, T	Broad-spectrum
	Nystatin	T	Yeasts
	Natamycin	T	Broad-spectrum
Other	Griseofulvin	O	Dermatophytes
	Amorolfine	T	Dermatophytes, <i>Candida</i> spp.
	Butenafine	T	Dermatophytes
	Ciclopirox	T	Dermatophytes, yeasts
	Haloprogin	T	Dermatophytes, <i>Candida</i> spp.
	Tolnaftate	T	Dermatophytes
	Undecylenic acid	T	Dermatophytes

^aO, oral; IV, intravenous; T, topical.

^bBroad-spectrum: dermatophytes, yeasts, *Aspergillus*, dimorphic fungi.

^cYeasts: *Candida* spp., *Cryptococcus neoformans*, *Malassezia pachydermatis*.

^dDimorphic fungi: *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Coccidioides immitis*, and *Sporothrix schenckii*.

^eMany other topical imidazoles such as bifonazole, butoconazole, oxiconazole, sulconazole, terconazole, and tioconazole are available for topical use.

of fungi, as with bacteria, is not always predictable. Both acquired and constitutive resistance have been described. In order to be useful clinically, in vitro susceptibility testing should reliably predict clinical outcome of therapy. Many factors may affect this outcome, including drug pharmacokinetics, drug interactions, host immune response, patient management, and virulence of the infecting microorganism. Because so many factors can affect the outcome of antifungal therapy, a low MIC does not necessarily predict clinical success. Similarly, a report that indicates that a fungus is resistant to an antifungal agent does not always mean that the use of that antifungal agent will result in an unfavorable clinical outcome. These discrepancies between in vitro results and clinical outcome are an ongoing problem, and have led to the establishment of a CLSI subcommittee to address standardization of testing methods and the development of interpretive criteria for antifungal drugs. Many recent studies have provided evidence that in vitro antifungal susceptibil-

ity tests correlate with the outcome of therapy in human medicine (Rex and Pfaller, 2002). In the absence of specific veterinary criteria, the standards developed in human medicine may be useful.

Antifungal Drug Resistance

Antifungal drug resistance can be intrinsic or acquired. Resistance can occur via selection of intrinsically resistant strains following the use of a drug in a particular environment, or via mutation of a susceptible strain which renders it resistant. Resistance following antifungal drug use is well recognized. The precise mechanism associated with resistance depends on the mode of action of the class of antifungal drug, and includes reduced drug uptake, drug export through efflux pumps, or reduced affinity of target enzymes. Unlike bacterial cells, intact fungal cells do not readily take up exogenous DNA. As a result, transferable drug resistance has not been described among widely divergent fungal taxa, and the spread of resistance has been

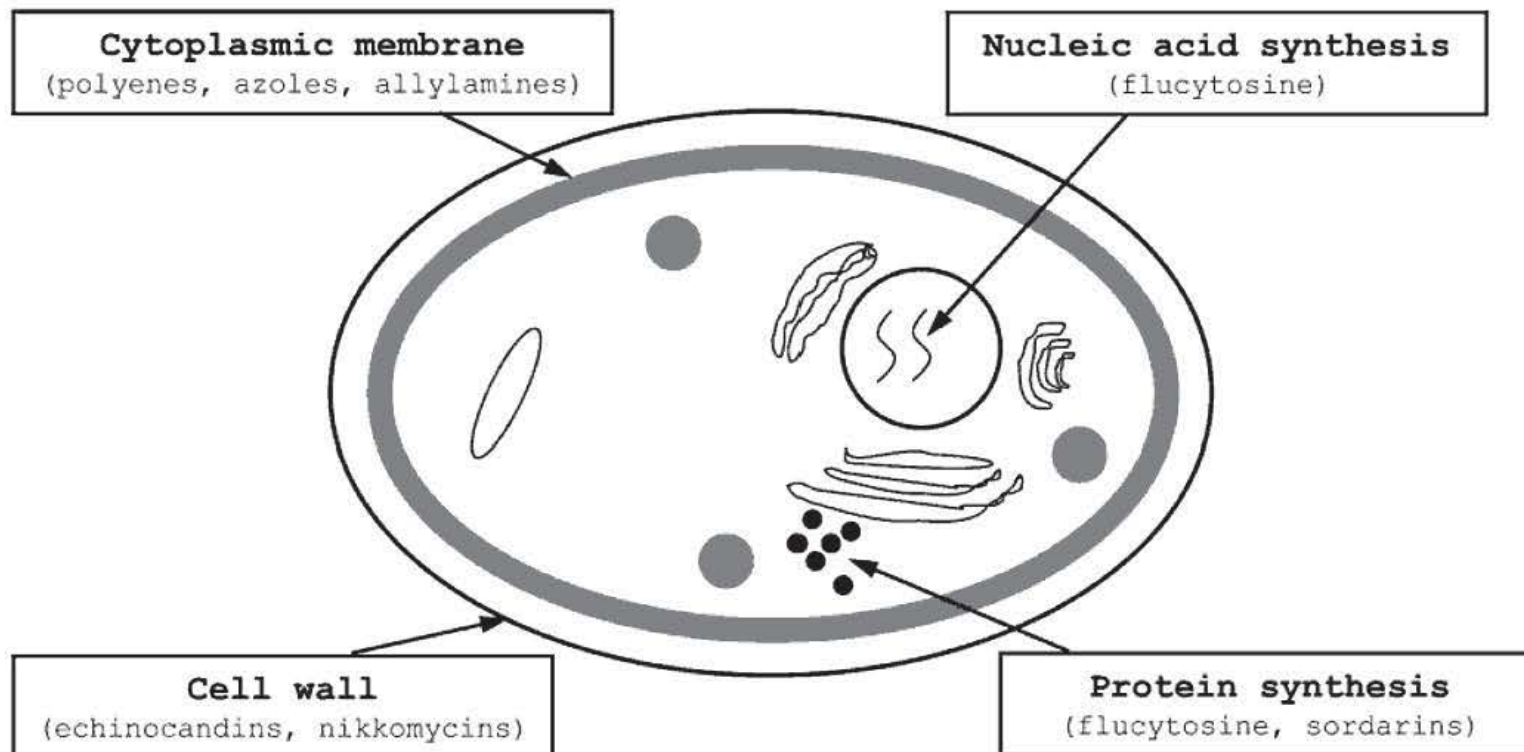


Figure 19.1. Action of antifungal agents on the fungal cell.

considerably slower than that observed in bacteria. Prevention of emergence and spread of resistant fungi depends on taking maximal advantage of the pharmacodynamic properties of the particular drug class, on the use of local rather than systemic treatment (thus reducing general exposure of the animal's fungal flora to antifungal agents), and on hygienic precautions. Combination antifungal therapy to reduce the emergence of resistance is a well recognized strategy to prevent emergence of flucytosine resistance.

Pharmacodynamics of Antifungal Agents

In vitro and laboratory animal studies have begun to define the pharmacodynamic characteristics of antifungal agents. Analysis of clinical data in humans also suggests that pharmacodynamic targets identified in animal models are predictive of outcome in humans (Andes, 2004). Polyenes and echinocandins exert a long post-antifungal effect and are concentration-dependent. The best predictor of efficacy for these drugs is a C_{max}:MIC ratio between 3:1 and 10:1, with high ratios conferring better activity. In contrast, flucytosine has a short post-antifungal effect and the best predictor of efficacy is the time period for which serum concentrations exceed the MIC of a given pathogen. The triazoles exert characteristics of both time- and concentration-dependent activity. The best predictor of efficacy for these drugs is a 24-hour area under the serum concentration versus time curve (AUC)/MIC ratio of 25:1.

Antifungal Drugs for Systemic Administration

Allylamines: Naftifine, Terbinafine

Naftifine is used topically to treat dermatophyte infections, while terbinafine is available for both oral and topical use in human medicine. Terbinafine is used in the treatment of dermatophytic, *Malassezia*, and *Sporothrix schenckii* infections, and there is interest in it for its activity against *Candida*, dimorphic, and filamentous fungi. It is used in people in the systemic treatment of persistent or intractable dermatophyte infections, in which it is more effective than ketoconazole, itraconazole, or griseofulvin.

Mechanism of Action

Allylamines are synthetic drugs which inhibit ergosterol biosynthesis by their effect against an important

enzyme (squalene epoxidase) involved in its synthesis. This effect causes fungal cell death primarily due to increased membrane permeability mediated by the accumulation of high concentrations of squalene, rather than by ergosterol deficiency.

Antifungal Activity

Isolates with a MIC ≤ 1 $\mu\text{g/ml}$ to terbinafine are considered susceptible, 2 to 4 $\mu\text{g/ml}$ represents intermediate susceptibility, and isolates with a MIC ≥ 8 $\mu\text{g/ml}$ are resistant. The MIC of terbinafine is low in vitro against dermatophyte species and a broad-spectrum of non-dermatophyte organisms, including *Aspergillus* spp., *Scopulariopsis brevicaulis*, *Sporothrix schenckii*, *Blastomyces dermatidis*, *Histoplasma capsulatum*, *Coccidioides immitis*, *Malassezia* spp., *Cryptococcus neoformans*, and certain *Candida* spp. The fungicidal activity of terbinafine offers a considerable advantage over many other antifungal agents.

Resistance

The prevalence and mechanisms of intrinsic or acquired resistance to terbinafine have not been well studied. Some studies have shown that acquisition of efflux-mediated multidrug resistance results in resistance to terbinafine. Other studies have also demonstrated several single-point mutations resulting in terbinafine resistance without cross-resistance to other antifungal agents.

Pharmacokinetic Properties

Terbinafine is a lipophilic allylamine compound which is well absorbed (> 70% in people) and binds strongly and non-specifically to plasma proteins. The absorption characteristics are not altered when terbinafine is taken with food. Terbinafine penetrates keratinized tissues, and enters the stratum corneum and sebum by direct diffusion through the dermis and living epidermis. Plasma concentrations are not particularly good indicators of concentrations in the target organs since the drug persists in the skin for prolonged periods of time. In a study in cats, there was no difference in plasma concentrations between low (10 to 20 mg/kg q 24h) versus high dose (30 to 40 mg/kg q 24h) of terbinafine. In contrast, concentrations in hair were significantly greater with the high dose (Kotnik et al., 2001). The excretion of terbinafine in the urine and feces is 80% and 20%, respectively, in people.

Table 19.2. Usual dosages of selected systemic antifungal agents in domestic animals.

Species	Drug	Dosage (mg/kg)	Route	Interval (h)
Dog/cat	Terbinafine	30	PO	24
	Amphotericin B (conventional)	0.5(dog); 0.25(cat)	IV ^a	3 x/week
	Amphotericin B (lipid)	2-3 (dog); 1 (cat)	IV ^a	3 x/week
	Flucytosine	50-75	PO	6-8
	Ketokonazole	10	PO	12
	Itraconazole	5	PO ^c	12-24
	Fluconazole	5-10	PO	12-24
	Griseofulvin (micro size)	50	PO	24
	Griseofulvin (ultramicro size)	10	PO	24
Horses	Amphotericin B (conventional)	0.3-0.9	IV [*]	24-48
	Ketoconazole	30 (in 0.2 N HCl)	NGT ^b	12
	Fluconazole	5 ^d	PO	24
	Itraconazole	5	PO ^c	12-24

^aDiluted to 1 mg/ml in 5% dextrose and administered over 1 to 2 h.

^bNasogastric intubation is required to avoid the irritant effect of HCl on the oral cavity and throat.

^cThe bioavailability of the oral suspension is superior to that of the capsules.

^dA loading dose of 14 mg/kg is recommended.

^{*}Diluted to 1 mg/ml in 5% dextrose and administered over 1-2 h.

Drug Interactions

There are no drugs that are contraindicated with terbinafine. In vivo studies have shown that terbinafine is an inhibitor of cytochrome P₄₅₀. Co-administration of terbinafine with drugs predominantly metabolized by the cytochrome P₄₅₀ 2D6 isozyme should be done with careful monitoring and may require a reduction in dose of the 2D6-metabolized drug. Terbinafine clearance is increased 100% by rifampin, a cytochrome P₄₅₀ enzyme inducer, and decreased 33% by cimetidine, a cytochrome P₄₅₀ enzyme inhibitor. Terbinafine clearance is unaffected by cyclosporine.

From a theoretical point of view, combinations of azoles and terbinafine should exhibit synergy since they are acting at different points of the same pathway. This has been corroborated in several studies in vitro. Combinations of terbinafine with fluconazole, itraconazole, or voriconazole have shown synergy in vitro against species of *Candida*, *Aspergillus*, *Mucorales* and even against fluconazole-resistant *Candida* isolates and itraconazole-resistant *Aspergillus* strains (Cuenta-Estrella, 2004).

The interaction of terbinafine and amphotericin B or flucytosine has also been assessed. In vitro studies have indicated that these combinations exhibit no interactions or are antagonistic against *Aspergillus* and other fungi.

Toxicity and Adverse Effects

Terbinafine is well tolerated with a low incidence of adverse reactions in dogs and cats. Adverse effects involve the gastrointestinal system and the skin. Abnormalities in liver enzymes and hematologic parameters are rarely observed in people.

Administration and Dosage

Dosage is summarized in Table 19.2.

Clinical Applications

Due to its high rate of efficacy, low incidence of adverse reactions and ability to achieve clinical success after a relatively short course of therapy compared to other agents, terbinafine is often the treatment of choice for various dermatomycoses in people. Terbinafine therapy has also been efficacious in some patients with sporotrichosis, aspergillosis, chromoblastomycosis, and leishmaniasis. There is also evidence that resistant *Candida* infections may respond to a combination of terbinafine and a triazole.

Terbinafine is more active in vitro than griseofulvin against *Microsporum canis*, *M. gypseum*, and *Trichophyton mentagrophytes* (Hofbauer et al., 2002). Terbinafine has been shown to be effective for the treatment of both experimental and naturally acquired dermatophytosis in dogs and cats. The length

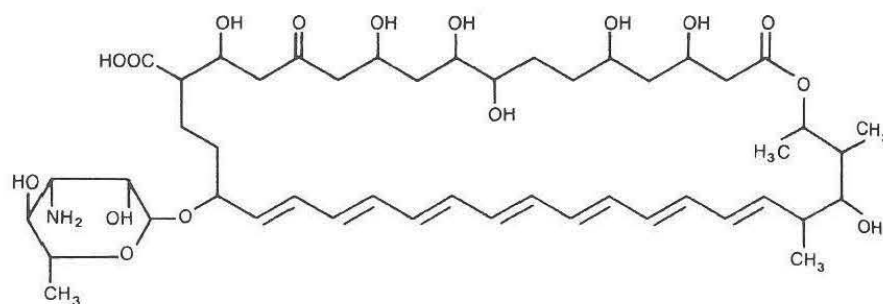


Figure 19.2. Structural formula of amphotericin B.

of therapy for mycological cure has ranged between 33 and 63 days (Kotnik et al., 2002; Moriello, 2004). Terbinafine has also been at least as effective as ketoconazole in reducing yeast counts in dogs with *Malassezia* dermatitis (Rosales et al., 2005).

Polyenes: Amphotericin B

The polyene group of antifungal agents includes amphotericin B and nystatin. Amphotericin B is used for systemic administration whereas nystatin is used topically. Amphotericin B was the mainstay of systemic antifungal treatment for many years. Although its place in systemic treatment of yeast or dimorphic fungal infections is challenged by the azole antifungal drugs, it is still the mainstay for systemic treatment of filamentous fungal infections. A major advantage of this drug is its fungicidal nature, so that it is often used in treatment of life-threatening yeast or dimorphic fungal infections. Its toxicity has been circumvented in recent years by development of lipid formulations which, though expensive, are coming into clinical use in animals.

Chemistry

Amphotericin is a heptaene product of *Streptomyces nodosus* belonging, like nystatin, to the polyenes (Figure 19.2). It is an amphoteric polyene macrolide that is poorly soluble in water and unstable at 37°C. The antifungal effects of the antibiotic are maximal between pH 6.0 and 7.5 and decrease at low pH. The amphotericin B sodium deoxycholate compound with phosphate buffer is more water soluble and is used for IV administration. Lipid-based formulations (liposomal [AmBisome®], colloidal [Amphocil® or Amphotec®], or lipid complex [Abelcet®]) are less toxic than the micellar suspension which is the conventional formulation (Fungizone®).

Mechanism of Action

Amphotericin B binds to ergosterol, the principal sterol of the fungal cell membrane, causing leakage of cell contents. The drug binds cholesterol in mammalian cell membranes less avidly, but its ability to bind to mammalian cells makes this the most toxic of the clinically useful systemic antifungal drugs.

Antimicrobial Activity

Amphotericin B is a broad-spectrum antifungal antibiotic with the advantage of generally fungicidal activity against most pathogenic fungi. Isolates with a MIC ≤ 1 $\mu\text{g/ml}$ are considered susceptible, 2 $\mu\text{g/ml}$ represents intermediate susceptibility, and isolates with a MIC ≥ 4 $\mu\text{g/ml}$ are resistant. *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Cryptococcus neoformans*, *Candida* spp., *Coccidioides immitis*, and *Sporothrix schenckii* are susceptible, in decreasing order (Table 19.3). Some resistant *Candida*, *C. immitis*, and *Mucor* have been described. Strains of filamentous fungi, although commonly susceptible, can vary from extreme susceptibility to resistance. Of the filamentous fungi, *Aspergillus* spp. are the most frequently resistant, although most are susceptible. McDonald et al. (1980) reported half the yeasts isolated from bovine mastitis to be susceptible. *Prototheca*, an algae associated with cutaneous, subcutaneous, mammary, and systemic infections in several species, is also susceptible. Lipid-based formulations have similar activity to the conventional preparation.

Resistance

Although rare, development of resistance during treatment of susceptible fungi such as *Candida* spp. has been described. *Pseudoallescheria boydii*, *Trichosporon beigelii*, and some dematiaceous fungi are intrinsically resistant to amphotericin B.

500 ml in dogs) and cats (same dose, in 400 ml) two to three times weekly was described as a way of administering large quantities of amphotericin without producing the marked azotemia associated with bolus IV injection (Malik et al., 1996a). The drug was administered in combination with a triazole drug for the treatment of cryptococcal infection.

Treatment duration with conventional amphotericin B varies with clinical response but may be up to 12 weeks. For blastomycosis the total cumulative dose used is about 12 mg/kg.

Clinical experience with lipid-based formulations in animals is limited, but dosages of 2 to 3 mg/kg three times weekly for a total of 9 to 12 treatments (cumulative dose of 24 to 27 mg/kg) have been used in dogs. In cats, a lower dose of 1 mg/kg three times weekly for a total of 12 treatments (cumulative dose of 12 mg/kg) has been recommended (Grooters et al., 2003). The advantages of lipid-based formulations may be offset by their high cost.

Clinical Applications

Amphotericin B is the most toxic antibiotic in clinical use, but its fungicidal action makes it the drug of choice for most systemic fungal infections (*Candida*, *Blastomyces*, *Coccidioides*, *Histoplasma*) in immunocompromised hosts. In noncompromised hosts, the less toxic, though fungistatic, ketoconazole and the newer triazole drugs may be equally valuable for yeast infections. Comparative clinical trials in veterinary species are required to support this statement. For systemic infections caused by dimorphic fungi, amphotericin B may be combined with, preceded, or be replaced in non-immunocompromised hosts by ketoconazole or itraconazole treatment. In a retrospective study of 115 dogs with blastomycosis, treatment with itraconazole was as effective as treatment with a combination of amphotericin B and ketoconazole (Arce-neaux et al., 1998). Amphotericin B lipid complex (Abelcet) has been used to treat dogs with blastomycosis at 1 mg/kg q 48 hours, for a total cumulative dose of 8 to 12 mg/kg. Most dogs given a cumulative dose of 12 mg/kg became clinically free of blastomycosis; the two dogs in the study receiving a total dose of 8 mg/kg had a relapse of blastomycosis. No dogs developed evidence of renal damage (Krawiec et al., 1996).

Amphotericin B was the only reliable antifungal drug for systemic aspergillosis and zygomycosis (*Mucor*, *Rhizopus*), but itraconazole and voriconazole

are challenging amphotericin's use for this purpose. Aerosol treatment of pulmonary aspergillosis may be one way to assure high lung levels and low toxicity. Amphotericin B has not always been effective in nasal or disseminated *Aspergillus* infections in animals, possibly because of their lack of susceptibility. Amphotericin B may be a drug of choice in the treatment of *Prototheca* infections. Lipid formulations of amphotericin B have been successfully used for the treatment canine leishmaniasis, but relapses have been reported (Lamothe, 2001; Cortadellas, 2003).

In horses, amphotericin B is not suitable for the local treatment of mycotic keratitis because of its poor activity against some filamentous fungi and its locally irritating nature. There are several reports of intralesional or systemic use of amphotericin B in horses. The drug was used successfully in treating localized cutaneous phycomycoses (Florida horse leech), in regimens that lasted up to six weeks (McMullan et al., 1977). A wide range of doses and administration protocols have been used for systemic administration (Table 19.2). In one report, pulmonary cryptococcosis was successfully treated with daily infusions of 0.5 mg/kg amphotericin B for a month.

Pyrimidine Synthesis Inhibitors: Flucytosine

Flucytosine (or 5-fluorocytosine) is a fluorinated pyrimidine, a low-molecular-weight compound slightly soluble in water but readily soluble in alcohol. It is the only available antifungal agent acting as an anti-metabolite.

Mechanism of Action

After permease-mediated entry into the fungal cell, flucytosine is deaminated to 5-fluorouracil, which is incorporated into messenger RNA. This perverted mRNA functions poorly, garbling codon sequences and producing faulty proteins. Conversion of 5-fluorouracil to 5-fluorodeoxyuridine monophosphate, on the other hand, causes inhibition of thymidylate synthase, which functions in fungal DNA synthesis and nuclear division.

Antifungal Activity

Flucytosine has a narrow spectrum of antifungal activity, being active against most *C. neoformans*, 80 to 90% of *Candida*, and most *Torulopsis*. The majority of yeast isolates from bovine mastitis are resistant. While a few *Aspergillus* strains are susceptible, dermatophytes, other filamentous fungi, and dimorphic fungi are re-

sistant. An MIC ≤ 4 $\mu\text{g/ml}$ is considered susceptible, 8 to 16 $\mu\text{g/ml}$ intermediate and MIC ≥ 32 $\mu\text{g/ml}$ is considered resistant. The drug is fungicidal at concentrations five times the MIC.

Resistance

About 10 to 20% of *Candida* spp. but only 1 to 2% of *C. neoformans* show resistance. However, resistance develops readily in vitro and in vivo: Up to two-thirds of fungal isolates change from susceptible to resistant during treatment. Because of this, flucytosine should never be used as a single agent, but rather, always in combination with other antifungal agents.

Pharmacokinetic Properties

Flucytosine is well absorbed from the intestine after oral administration in humans, giving peak plasma concentrations of 70 to 80 $\mu\text{g/ml}$ one to two hours after a dose of 37.5 mg/kg (Bennett et al., 1979). Half-life in humans is about four hours; in the presence of renal impairment the half-life is increased. Penetration into tissues, including CSF, is excellent. The drug is largely excreted in the urine by glomerular filtration in unchanged form.

Drug Interactions

Combination with amphotericin B is commonly synergistic, because amphotericin B increases fungal permeability to flucytosine. Combination of flucytosin with amphotericin B or with an azole is superior to amphotericin B or azole monotherapy for the treatment of cryptococcosis.

Toxicity and Adverse Effects

Flucytosine is well tolerated. Occasional side effects reported are reversible anorexia, nausea, vomiting, diarrhea, mild elevations of liver enzymes, and bone marrow depression resulting in leucopenia. Skin eruptions characterized by depigmentation, followed by ulceration, exudation and crust formation have been reported in dogs (Malik et al., 1996b). Lesions resolve following discontinuation of therapy.

Administration and Dosage

Dosage is summarized in Table 19.2. The drug is given in capsule form at a dosage of 150 to 225 mg/kg daily in three or four divided doses. Flucytosine should always be used in conjunction with amphotericin B or an azole.

Clinical Applications

The major application is in the treatment of cryptococcal infection in cats. Its use for this purpose has now largely been replaced by triazole drugs. The drug should be combined with amphotericin B or an azole to prevent the emergence of resistant mutants. The usual dose of amphotericin B can be reduced to half or less, or the normal dose may be administered for a shorter period. Ketoconazole can substitute for amphotericin B and significantly reduces the length of treatment required with either drug alone (Shaw, 1988). The use of other azole drugs has shown the same effect, experimentally. There are two reports, Moore (1982) and Wilkinson et al. (1983) that describe the successful treatment of cryptococcosis in cats treated with flucytosine as a single agent. However, because of the likelihood of resistance, this is not recommended.

Azoles: Imidazoles and Triazoles

Imidazole and triazole drugs have the common antifungal action of inhibiting cytochrome P₄₅₀-dependent ergosterol synthesis, leading to disruption of fungal membranes and membrane-bound enzymes, as well as other antifungal effects. All are considered fungistatic drugs, but at high concentrations some azoles may have a fungicidal effect. Because of the potential to be fungistatic all treatment regimens should be prolonged, especially in immunocompromised patients.

Azole drugs were first extensively evaluated in the early 1970s for their antifungal activity. Two imidazoles, clotrimazole and miconazole, are effective topical antifungal agents. Neither can be used parenterally: Clotrimazole rapidly induces hepatic inactivating enzymes, and the toxicity of the solubilizing agent required for IV administration of miconazole limits its use. Another imidazole, ketoconazole, was developed in the late 1970s and became a major addition in antifungal therapy. It has a broad antifungal spectrum, can be administered orally, and is relatively non-toxic. Further development of the azoles, e.g., substitution of the imidazole ring by a triazole ring, produced compounds such as fluconazole, itraconazole and voriconazole (Figure 19.3). These products all have greatly increased half-lives, increased bio-availability following oral administration, lower toxicity, and enhanced antifungal activity compared to many of the imidazole drugs.

Table 19.3. In vitro activity (MIC₉₀, µg/ml) of selected systemic antifungal agents against common fungi.

Organisms	Amphotericin B	Flucytosine	Ketoconazole	Fluconazole	Itraconazole	Caspofungin
Filamentous fungi						
<i>Aspergillus fumigatus</i>	1	>64	16	>64	0.25	0.06
<i>A. flavus</i>	1	>64	8	>64	0.25	0.06
<i>Cladosporidium</i> spp.	0.5	>64	16	32	0.125	
<i>Mucor</i> spp.	0.25	>64	>64	>64	4	>16
<i>Rhizopus</i> spp.	0.25	>64	>64	>64	2	>16
Yeasts						
<i>Candida albicans</i>	0.5	0.125	0.125	0.25	0.03	1
<i>C. glabrata</i>	1	0.125	>64	>64	>16	1
<i>C. tropicalis</i>	1	0.25	0.06	0.5	0.25	1
<i>Cryptococcus neoformans</i>	0.5	8	0.25	2	0.008	>16
Dimorphic fungi						
<i>Blastomyces dermatidis</i>	0.5		0.5	32	0.125	8
<i>Coccidioides immitis</i>	0.25	>64	0.5	4	0.125	32
<i>Histoplasma capsulatum</i>	0.25	>64	0.25	2	0.06	4
<i>Sporothrix schenckii</i>	4	>64	4	>64	4	>16

Pharmacokinetic Properties

Amphotericin B is not absorbed well orally, and parenteral (IV) administration is required. The half-life in dogs after IV injection of conventional amphotericin B is about 26 hours (Kukui et al., 2003). The drug is thought to bind to plasma or cellular lipoproteins and to be released slowly from these sites. Only about 5% of the injected dose is excreted in the kidneys, but the agent continues to be excreted in the urine of humans for several weeks after therapy is stopped. Penetration into cerebrospinal fluid (CSF) is poor (5%) but increases in meningitis. Absorption from the lungs following aerosol administration is poor. Because of the poor systemic absorption following aerosol administration this route has been used successfully in the treatment of pulmonary aspergillosis. Characteristic of lipid-based formulations is their reduced volume of distribution, which allows greater drug concentration in serum. This is probably the result of decreased interaction of the amphotericin with host proteins or membrane cholesterol. Lipid-based formulations appear to be taken up extensively by the reticulo-endothelial system, which may give them significant therapeutic advantage. The lipid complex but not the liposomal or conventional amphotericin B are concentrated and accumulate in lung tissue (Matot and Pizov, 2000). This affinity for the lung may have implications in the treatment of fungal pneumonia.

Drug Interactions

Because of both the serious nature of systemic fungal infections and the toxicity of amphotericin B, considerable effort has been expended to find synergistic combinations of drugs that will reduce dosage and speed cure.

Flucytosine and amphotericin B show additive or synergistic effects in vitro against *Candida*, *Cryptococcus*, and *Aspergillus*. Flucytosine reduces the concentration of amphotericin B necessary to inhibit growth of *Candida* and *Cryptococcus* in vitro. The combination is synergistic in cryptococcal meningitis in humans, producing faster cure, fewer relapses, more rapid sterilization of CSF, and less nephrotoxicity.

There is a theoretical concern that amphotericin B and azole agents will lead to antagonism because there will be less ergosterol in the cell membrane available to the polyene, as a result of the azole inhibiting ergosterol synthesis. Amphotericin B can also interfere with the influx of the azole agents by damaging the membrane structure. Combination with various imidazoles or triazoles against *Candida* spp., *C. neoformans*, and *Aspergillus* spp. has produced complex interactions in vitro that are hard to interpret. Results of animal models of candidiasis have given contradictory results, with most studies showing either indifference or antagonism. In contrast, a clinical trial of invasive candidiasis in people revealed a significant advantage of

the combination fluconazole-amphotericin B over fluconazole monotherapy (Rex et al., 2003). Results in animal models of invasive aspergillosis and cryptococcal infection have given equivocal results with some studies showing synergism, some showing antagonism, and most showing indifference (Cuenca-Estrella, 2004). Combination with ketoconazole has been used successfully to treat systemic mycoses in dogs (Richardson et al., 1983), but confirmatory follow-up studies are unavailable.

Toxicity and Adverse Effects

Renal toxicity inevitably accompanies treatment with micellar (conventional) amphotericin B. In humans, the damage is reversible when the total dose is below 4 g. Monitoring of blood urea nitrogen (BUN) or creatinine shows the extent of renal damage, which can be reversed either by temporarily stopping treatment or by decreasing the dosage. Dosing every other day reduces nephrotoxic effects compared to administering the same dose daily (Butler and Hill, 1964). Other side effects include thrombophlebitis at the injection site and hypokalemia with resulting cardiac arrhythmias, sweating, nausea, malaise, and depression. In dogs and cats, signs of nephrotoxicity develop within three or four weeks of starting treatment, associated with BUN levels of 60 to 70 mg/dl. The effect is reversible and the drug should be discontinued until BUN falls below 40 mg/dl. Blood urea nitrogen should be monitored twice weekly during treatment. In addition, serum potassium should be monitored and hypokalemia corrected by oral supplementation. Hypokalemia does not seem to be as common in dogs and cats as in humans. Concurrent use of flucytosine decreases the dosage required to treat cryptococcal infection.

Lipid-based formulations reduce the toxicity of amphotericin B, reducing the infusion-related toxicities (nausea, fever, chills) and markedly reducing nephrotoxicity. Because of reduced toxicity, daily doses of the lipid-based formulations range in humans up to 3 to 5 mg/kg daily compared to 0.5 to 1 mg/kg q48 hours for the conventional form. In a recent meta-analysis of the human literature, lipid formulations conferred a significant advantage over conventional amphotericin B in terms of reduced risk of mortality and renal toxicity (Barrett et al., 2003).

Doses higher than 5 mg/kg of conventional amphotericin B in dogs resulted in death as a result of cardiac abnormalities. Doses of 2 to 5 mg/kg occasionally

caused cardiac arrhythmias in dogs, but doses less than 1 mg/kg were without effect on the heart. Administration of the liposomal formulation to dogs at daily dosages of 8 and 16 mg/kg resulted in weight loss, vomiting and tubular necrosis. A daily dose of 4 mg/kg for 30 days was associated with occasional vomiting, a moderate increase in BUN and creatinine concentrations and histopathologic changes consistent with moderate tubular nephrosis. In contrast, a daily dose of 1 mg/kg was well tolerated and was only associated with an increased urine volume and lower specific gravity (Bekerski et al., 1999). Renal and clinicopathologic changes observed with administration of the liposomal formulation at a daily dose of 4 mg/kg were similar to those reported after administration of the colloidal formulation at a dose of 5 mg/kg or to the conventional amphotericin B formulation administered at a daily dose of 0.6 mg/kg.

Administration and Dosage

Dosage is summarized in Table 19.2. There is no general agreement as to the optimum dosage, total dose, or duration of treatment required for amphotericin B. The dosage used for the conventional formulation has ranged between 0.25 to 1.0 mg/kg/day; it is lower in patients with normal defenses and more susceptible organisms.

For otherwise healthy animals, IV dosage is 0.5 mg/kg q48 h; BUN is monitored for evidence of kidney damage. On the first day the total dose is diluted in 20 ml of 5% dextrose and 5 ml given; if no acute anaphylactic response develops, in 1 minute, the remainder is given over 45 seconds. Thereafter the total dose is given over one minute in 20 ml of 5% dextrose. If BUN exceeds 60 mg/dl, the dose is discontinued or reduced 25 to 50% until BUN falls below 40 mg/dl. Administration by slow IV infusion, in 1 L of 5% dextrose over five hours, though inconvenient, is preferable because it causes less severe systemic toxicity (vomiting, diarrhea, weight loss) and less renal damage (Legendre et al., 1984; Rubin et al., 1989). In severely debilitated dogs an initial dosage is 0.2 mg/kg IV, increasing by 0.1 mg/kg daily until day four (0.5 mg/kg), then using this maintenance dosage as described. In cats with cryptococcal infection, combination with flucytosine reduces the time required for successful treatment.

Subcutaneous administration of amphotericin in 0.45% saline with 0.5% dextrose (0.5 to 0.8 mg/kg in

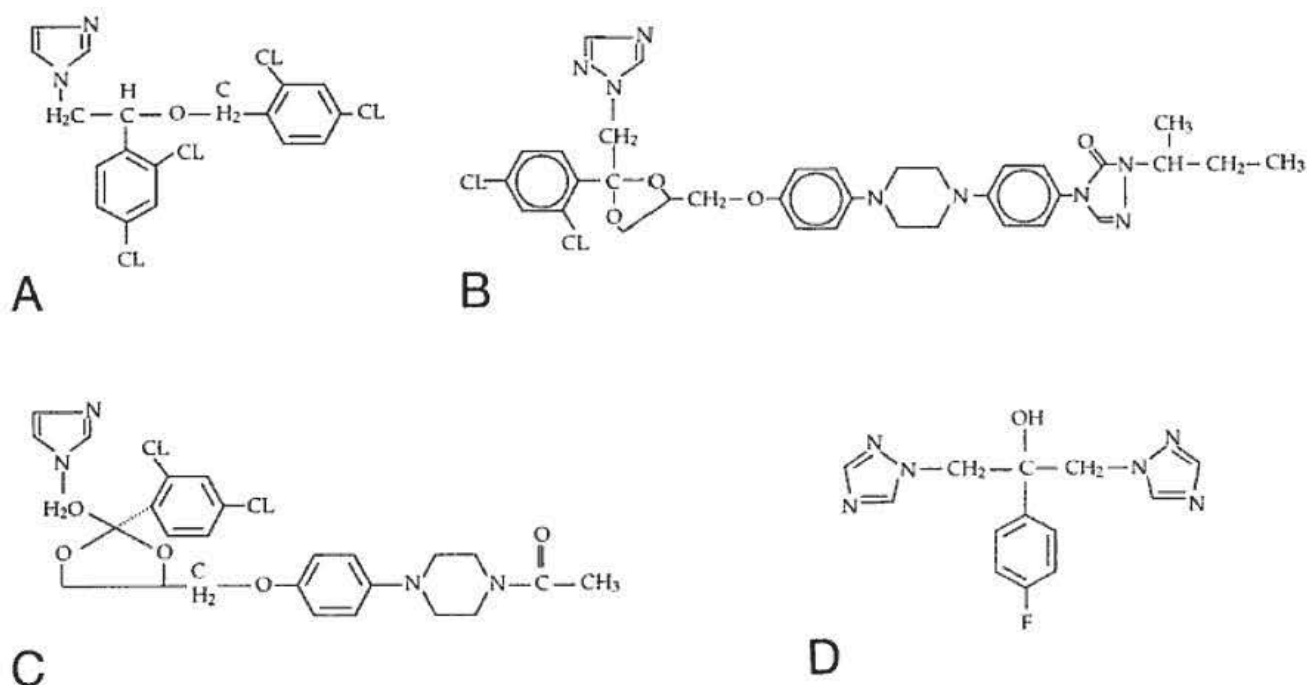


Figure 19.3. Structural formulas of representative antifungal azole compounds: A, miconazole; B, itraconazole; C, ketoconazole; D, fluconazole.

Drug Interactions

The azoles may be associated with three types of drug interactions. First, because azoles are important inducers of the CYP3A4 enzyme system, they may slow the metabolism of drugs that are metabolized by the CYP pathway, increasing these drugs' plasma concentrations. Second, drugs that are CYP inducers can speed the metabolism of the azoles, thereby lowering their plasma concentrations. Third, when an azole is given concurrently with some drugs, there may be two-way interactions in which the azole can raise the serum level of a concomitant drug and the concomitant drug can lower the concentration of azole.

Imidazoles: Ketoconazole

Chemistry

Ketoconazole is a poorly water-soluble, highly lipophilic, weak dibasic compound that requires an acid pH for dissolution, which precedes absorption from the stomach. There are conflicting reports on the effect of feeding on absorption of the drug.

Antimicrobial Activity

Ketoconazole is generally fungistatic against a wide range of filamentous fungi, including dermatophytes,

yeasts, and dimorphic fungi (Table 19.3). Isolates with a MIC ≤ 0.125 $\mu\text{g/ml}$ are considered susceptible, 0.25–0.5 $\mu\text{g/ml}$ represent intermediate susceptibility, and isolates with a MIC ≥ 1 $\mu\text{g/ml}$ are resistant. Most isolates of *C. albicans* are susceptible but *C. tropicalis* are resistant. *Malassezia pachydermatis* isolates are susceptible. The drug has favorable in vitro activity against *H. capsulatum*, *C. immitis*, and *B. dermatidis*. *Aspergillus* spp., *Fusarium* spp., but the *Zygomycetes* group of fungi are resistant. Ketoconazole is active against some Gram-positive bacteria, and the drug has activity against *Leishmania*, *Plasmodium*, and other protozoa. The in vitro resistance of *Prototheca* is apparently contradicted by in vivo response to ketoconazole treatment. In early reports, about three-fourths of yeasts isolated from bovine mastitis were susceptible to ketoconazole and one-third to miconazole, compared to nearly complete susceptibility to clotrimazole (McDonald et al., 1980). Data from more recent studies are not available.

Resistance

Acquired resistance has been reported with ketoconazole but has not been well documented. This is due in part to the lack of standardized antifungal susceptibil-

ity tests. Hopefully, this issue will be addressed within the next few years, now that standardized methods have been published.

Pharmacokinetic Properties

Ketoconazole is well absorbed after oral administration. In dogs, plasma concentration after an oral dose of 10 mg/kg peaks at 8.9 µg/ml within one to two hours (Moriello, 1986). The drug requires an acid environment for full dissolution and absorption and should be given with food. Ketoconazole is extensively metabolized in the liver to inactive compounds, which are excreted in the bile. The distribution of ketoconazole is limited, and its penetration into CSF is minimal. The drug does, however, enter milk. Little active drug is excreted in urine. Administration of ketoconazole orally to adult horses at a dose of 30 mg/kg does not result in detectable serum concentrations. Administration of the same dose in 0.2 N HCl resulted in peak serum concentrations of 3.7 µg/mL and a bioavailability of only 23% (Prades et al., 1989).

Drug Interactions

Combination of amphotericin B with ketoconazole gives additive effects in the treatment of cryptococcal infection. Experimentally, however, ketoconazole antagonizes the activity of amphotericin against *Aspergillus*. Combination with flucytosine in the treatment of cryptococcal infections may prevent the emergence of resistance to flucytosine and reduce time required for treatment. The azoles may be associated with three types of drug interactions as indicated above.

Toxicity and Adverse Effects

Nausea, vomiting, dizziness, itching, and increases in liver enzyme levels are adverse effects in humans. In dogs the most common adverse effects are inappetence, pruritus, alopecia, and reversible lightening of the hair (Moriello, 1986). Long-term treatment of dogs (mean 13.6 months, range 3.5 to 37) has been associated with the development of cataracts (da Costa et al., 1996). The mean time from the initiation of treatment to development of cataracts was 15 months. Cats appear to be more susceptible to the toxic effects of ketoconazole and may develop anorexia, depression, weight loss, diarrhea, and fever. In a few human patients (1 in 15,000) severe hepatitis may develop. This reaction does not appear to be dependent on dose. High doses in dogs (greater than 80 mg/kg/day)

for prolonged periods have produced severe hepatitis (Moriello, 1986). Cats treated concurrently with flucytosine have shown evidence of liver damage and developed leukopenia, possibly because of additive or synergistic toxicity. Mammalian P₄₅₀ systems responsible for cholesterol, cortisol and testosterone synthesis are significantly inhibited. Gynecomastia, decreased libido, and azoospermia have been reported in a small percentage of men but not in dogs or cats. Ketoconazole at therapeutic dosage suppressed plasma cortisol and testosterone but increased progesterone concentrations in dogs (Willard et al., 1986a). Similar effects were not observed in cats (Willard et al., 1986b). Care should therefore be taken when using the drug in male breeding dogs. Ketoconazole may be embryotoxic and teratogenic and should not be given to pregnant animals.

Administration and Dosage

Dosage is summarized in Table 19.2. Absorption from the gastrointestinal tract may be erratic. The oral dosage of ketoconazole in dogs and cats for the treatment of ringworm was extrapolated from human clinical studies and varies from 5 to 10 mg/kg daily for four to six weeks. Recommended dosage of ketoconazole for systemic fungal infections in dogs and cats is 10 mg/kg q 12 h.

Clinical Applications

Ketoconazole was once the most widely used antifungal drug in veterinary medicine because of its efficacy, safety relative to amphotericin B, oral dosing, and cost. However, ketoconazole is now eclipsed by fluconazole and itraconazole, because of their greater activity, lower toxicity, and improved pharmacokinetic properties. Ketoconazole is now a second-line drug for the treatment of dimorphic fungi (candidiasis, cryptococcosis) and systemic mycosis (coccidioidomycosis, histoplasmosis, blastomycosis) in dogs and cats.

Ketoconazole is an attractive alternative to amphotericin B in the treatment of systemic mycotic infections (dimorphic fungi, candidiasis, cryptococcosis) in non-immunocompromised animals. Because of its fungistatic nature, it is recommended that therapy for systemic infections be prolonged (three to six months) to minimize the potential for relapses. Although ketoconazole has been used successfully alone in treatment of blastomycosis, coccidioidomycosis, cryptococcosis, and histoplasmosis, current recommendations are to

use it in conjunction with amphotericin B to treat these systemic mycoses. In the treatment of canine blastomycosis, Legendre et al. (1984) suggested that amphotericin B was better than ketoconazole (10 mg/kg daily), but that a course of amphotericin (total 4 mg/kg) followed by ketoconazole (10 mg/kg daily for two months) was as effective as more prolonged treatment with amphotericin (total 8 to 9 mg/kg) and produced less kidney damage.

Ketoconazole is not useful in zygomycosis and its efficacy against *Aspergillus* infections is questionable, in that only about 50% of dogs treated for nasal aspergillosis were cured by the use of ketoconazole alone (5 mg/kg, q12 hours) (Sharp and Sullivan, 1989). The combination of ketoconazole and 5-flucytosine reduced the dose and duration of treatment required for feline cryptococcosis compared to either drug alone (Shaw, 1988).

Ringworm in dogs and cats has been treated successfully with 10 mg/kg of ketoconazole daily for 10 to 20 days (DeKeyser and Van den Brande, 1983). Because of fewer adverse effects (especially in cats), lower cost, and greater efficacy in vitro, griseofulvin is preferred over ketoconazole for the treatment of ringworm. Animals with lesions may require six weeks (range four to ten) for complete resolution (Medleau and Chalmers, 1992). Ketoconazole is effective in the oral treatment of soft tissue sporotrichosis in humans, but high doses are required and relapses may occur. Ketoconazole has been the systemic treatment of choice for *Malassezia* infections, although topical treatment with ketoconazole or miconazole is more usual.

Triazoles: Itraconazole

Chemistry

Like ketoconazole, itraconazole is a poorly water-soluble, highly lipophilic, weakly dibasic compound that also requires an acid pH for absorption from the stomach. It is now available in both IV and oral formulations.

Antimicrobial Activity

Itraconazole is a potent inhibitor of most fungal pathogens of animals, because of its greater selectivity for the fungal cytochrome system than ketoconazole (Table 19.3). Itraconazole's spectrum includes dimorphic fungi, *Cryptococcus*, *Sporothrix*, *Alternaria*, most *Aspergillus*, *C. albicans*, and *C. tropicalis*, but activity

against other *Candida* spp., the dermatophytes, and the agents of phaeohyphomycoses is variable. While itraconazole is considered a fungistatic agent, it has been shown to be fungicidal at low concentrations against some fungi. Isolates with MIC ≤ 0.125 $\mu\text{g/ml}$ are regarded as susceptible, with MIC 0.25–0.5 $\mu\text{g/ml}$ intermediate, and ≥ 1 $\mu\text{g/ml}$ resistant.

Pharmacokinetic Properties

A lipophilic drug, itraconazole is well absorbed following oral administration and widely distributed to tissues (except the CSF), where it achieves concentrations several times those found in plasma. Skin concentrations exceed plasma concentrations and marked keratin binding occurs; this is significant in the treatment of dermatophyte infections. Food and an acidic environment significantly enhance absorption. It is cleared mainly by intra-hepatic metabolism and detectable concentrations do not appear in the urine or CSF, even though itraconazole has been successfully used in the treatment of cryptococcal meningitis. A steady-state serum concentration was achieved in cats after two to three weeks of administration of 10 mg/kg q 24 hours (Boothe et al., 1997). In horses and in cats, the oral suspension is better absorbed than the capsules. The half-life in horses is 6.5 h (Davis et al., 2005). As with other azoles, concurrent administration of rifampin will increase hepatic metabolism.

Toxicity and Adverse Effects

Toxicity reported in humans is minimal and limited to nausea in a small proportion of patients and rare, transient increases in hepatic enzymes. Blockage of adrenal steroid or testosterone synthesis has not been described. There were no adverse effects reported in cats treated with itraconazole at 10 mg/kg/day for three months compared to anorexia and weight loss in cats treated with the same dosage of ketoconazole (Medleau et al., 1990). Adverse effects reported in dogs and cats have, apart from occasional anorexia and vomiting, been minimal. Dosage can be progressively decreased in animals which vomit or become anorectic until these effects are no longer observed. Fatal hepatotoxicity was reported in one cat treated with over 20 mg/kg (Medleau et al., 1995). Cutaneous lesions suggestive of drug eruption have been described in a dog (Plotnick et al., 1997). Itraconazole is contraindicated in pregnancy.

Administration and Dosage

Recommended dosage is summarized in Table 19.2. Itraconazole is available in oral capsule, oral suspension, and intravenous formulations. The oral suspension is preferred to capsules in domestic animals because of enhanced bioavailability. Itraconazole, administered orally, preferably with food at a dose of 5 mg/kg q12-24 hours is recommended for dogs, cats, horses and other monogastric animals. Duration of treatment should be tailored to clinical and mycological response. For example, a dose of 5 mg/kg q24 hours for 60 days was as effective as 10 mg/kg q24 hours for the treatment of canine blastomycosis and associated with fewer adverse effects (Legendre et al., 1996); however, about 20% of treated dogs relapsed. A dosage of 5 mg/kg q12 hours for 60 days or more was used to treat histoplasmosis in cats; recurrence of disease occurred in two of eight treated cats, which required further treatment (Hodges et al., 1994). The dosage of 5 mg/kg q12 hours in cats can safely be increased to 10 mg/kg q12 hours (Boothe et al., 1997). Dosage of 1.5-3 mg/kg q24 hours, usually for 15 days (but sometimes for longer), was effective in controlling dermatophytosis in cats (Mancianti et al., 1998). The dosage in humans is ≤ 400 mg/day but higher doses (600 mg) have been used in infections that have not responded to the lower dose, although toxicity was observed in long-term use of high dosage (Sharkey et al., 1991).

Clinical Applications

Because of its potency, pharmacokinetic advantages, clinical efficacy, and safety, itraconazole has become the systemic treatment of choice for aspergillosis, blastomycosis, coccidioidomycosis, histoplasmosis, and sporotrichosis. It has similar application to ketoconazole but its broader spectrum includes *Aspergillus* and the agents of phaeohyphomycosis. It has greater activity than ketoconazole against *Sporothrix*. It is as effective as but less toxic than ketoconazole in the treatment of cryptococcosis and dermatophyte infections. It is as effective as griseofulvin in the treatment of dermatophyte infection in cats (Moriello and DeBoer, 1995). Treatment of serious infections with this generally fungistatic drug needs to be prolonged (three-plus months), and relapses anticipated. The effectiveness of treatment may be monitored by serology, for cryptococcal (Jacobs et al., 1997) and possibly for other systemic mycoses. In the treatment of

serious systemic mycoses, combination with amphotericin B in the initial stages of treatment is recommended.

Although itraconazole has been used successfully to treat disseminated *Aspergillus* infections in dogs (Kelly et al., 1995), its oral administration has been found to be ineffective in treatment of canine nasal aspergillosis. In high doses, it has been used to successfully treat cerebral aspergillosis in humans (Verweij et al., 1997). The drug has a particularly useful place in the systemic treatment of aspergillosis in pet birds; pharmacokinetic studies in Blue-fronted Amazon Parrots suggested that a dosage of 10 mg/kg q24 hours was appropriate (Orosz et al., 1996).

In horses, topically applied 1% itraconazole with 30% DMSO ointment gave considerably higher corneal concentrations than drug without DMSO (Ball et al., 1997a); applied every four hours for a median of 35 days, it was effective in resolving keratomycosis in the majority of cases (Ball et al., 1997b). Administered orally for 3.5 to 5 months, itraconazole was effective in the treatment of mycotic rhinitis in horses (Korenek et al., 1994). Oral administration of 5 mg/kg q24 hours was combined with locally applied enilconazole in the successful treatment of guttural pouch mycosis (Davis and Legendre, 1994).

Triazoles: Fluconazole

Fluconazole is a specific inhibitor of the fungal enzyme lanosterol 14 α -demethylase. This inhibition prevents the conversion of fungal cell lanosterol to the membrane lipid ergosterol. It is highly selective for fungal cytochrome P₄₅₀ enzymes.

Chemistry

Fluconazole is a water-soluble bis-triazole compound with marked pharmacokinetic differences from ketoconazole and itraconazole.

Antimicrobial Activity

Fluconazole has broad antifungal activity, including *Candida albicans* and many other *Candida* spp., *Cryptococcus neoformans*, *Coccidioides immitis*, and *Histoplasma capsulatum* (Table 19.3). Fluconazole has limited activity against *Blastomyces dermatitidis*. It is ineffective against *Aspergillus*. Organisms with MIC ≤ 8 μ g/ml are regarded as susceptible, with MIC 16-32 μ g/ml as intermediate, and ≥ 64 μ g/ml as resistant.

Resistance

Candida krusei is intrinsically resistant to fluconazole and as many as 15% of *C. glabrata* isolates may exhibit resistance. Progressive development of acquired resistance in *C. albicans* has been reported during long term treatment, particularly in immunosuppressed patients. Resistance may also develop when fluconazole is used to treat histoplasmosis.

Pharmacokinetic Properties

In contrast to other azoles, fluconazole is a water-soluble, weakly protein-bound drug, and oral absorption is unaffected by acid. It is well absorbed after oral administration and, because of its low molecular weight, water solubility, and lack of protein binding, distributes widely to tissues. Its ability to reach high concentrations (50 to 90% of serum) in CSF is a particular advantage in treating yeast (e.g., *Cryptococcus* infections) in the brain. Food does not affect absorption. It is excreted unchanged in the urine. Half-life in humans is 25 to 30 hours, so that single oral dosing is used for some types of infections. Half-life in cats has been reported as 14 (Malik et al., 1992) or 25 hours (Vaden et al., 1997). Half life in horses is 40 hours (Latimer et al., 2001). Oral bioavailability is 100% in both cats and horses. In contrast to ketoconazole, fluconazole can be administered IV.

Toxicity and Adverse Effects

Fluconazole is well tolerated after oral or IV administration, with minimal side effects other than nausea, skin rash, and headaches in some human patients. There is no evidence of interference with steroid biosynthesis but elevations in hepatic enzymes, which are usually mild, have been reported. It can interfere with the metabolism of drugs whose metabolism is dependent on hepatic P₄₅₀ enzymes.

Administration and Dosage

Fluconazole is available in both oral and intravenous formulations, although it is used almost exclusively orally in veterinary medicine. Dosage recommendations for fluconazole in animals are presented in Table 19.2. Cryptococcal infections in cats were successfully treated with 50 mg/cat q12 hours (Malik et al., 1992); in one animal 100 mg q12 hours was required. Pharmacokinetic considerations led Vaden et al. (1997) to suggest a dose in cats of 50 mg/cat q24 hours. A dose of 11 mg/kg q 24 hours was used in the

effective treatment of cryptococcosis in a dog. This dose was reduced to 4.2 mg/kg several weeks later, when anorexia developed (Tiches et al., 1998).

Clinical Applications

Fluconazole has had excellent success in oral treatment of local or systemic candidiasis in humans, and is a drug of choice for this purpose. In severe candidiasis, it may be combined with amphotericin. Fluconazole is also the treatment of choice for cryptococcal meningitis in AIDS patients. It is as effective as amphotericin B in treatment of acute cryptococcal meningitis in all patients and is more effective in maintenance therapy in AIDS patients. Initial concurrent treatment with amphotericin B is recommended. It is a drug of choice for candidal cystitis. In animals, fluconazole is probably the drug of choice for cryptococcal infections, for the systemic treatment of candidal infections, and for the treatment of coccidioidosis. It may be useful in the treatment of *Prototheca* infections.

Efficacy against blastomycosis, histoplasmosis and sporotrichosis in humans has been moderate at the dosages assessed. Fluconazole is not as effective as itraconazole for these infections. It has little in vitro activity against *Aspergillus* spp. and thus is not recommended for the treatment of aspergillosis. Paradoxically, fluconazole was administered PO at 2.5 to 5 mg/kg/day to dogs in a divided dose in the successful treatment of nasal aspergillosis or penicilliosis in six of ten dogs (Sharp et al., 1991).

Triazoles: Voriconazole

Voriconazole is the first licensed member of the second generation of triazoles which includes posaconazole and ravuconazole (see below). It is structurally related to fluconazole rather than to itraconazole.

Antimicrobial Activity

Voriconazole is active against a wide spectrum of medically important fungi, including dermatophytes, opportunistic yeasts (*Candida* spp, *Cryptococcus neoformans*), opportunistic filamentous fungi (*Aspergillus* spp., *Fusarium* spp.) and dimorphic fungi (*Histoplasma*, *Coccidioides*, *Blastomyces*, *Sporothrix*). Isolates with a MIC \leq 1 μ g/ml are considered susceptible. In contrast to fluconazole, voriconazole is active against most *C. krusei* and most *C. glabrata* isolates. However, cross-resistance of resistant *C. albicans* and *C. glabrata* strains has been reported. Voriconazole exerts time-

dependant fungicidal activity against *Aspergillus* in vitro. This process is slightly better than with itraconazole but slower than with amphotericin B, as would be expected from their respective mechanisms of action.

Pharmacokinetic Properties

Voriconazole is available in oral and IV formulations. It is extensively metabolized by the liver and, unlike fluconazole and amphotericin B, does not depend on renal function for excretion. However, the IV formulation contains sulfobutyl ether β -cyclodextrin sodium, which is excreted by the kidneys and tends to accumulate in patients with renal failure. Unlike itraconazole, voriconazole is not dependant on gastric acid for absorption and the drug is entirely absorbed in dogs (Roffey et al., 2003). Voriconazole has a high volume of distribution and excellent tissue penetration. In a guinea pig model CSF concentrations were about half that of plasma whereas brain tissue concentrations were two-fold higher.

Toxicity and Adverse Effects

Voriconazole is generally well tolerated. Visual disturbance is the most common adverse effect, occurring in 20 to 40% of human patients. The effect is dose-related and it is seldom necessary to stop therapy. This effect has not been described with other triazoles. Other adverse effects and drug interactions are similar to that reported with other triazoles.

Clinical Applications

Voriconazole is used in people for the treatment of invasive aspergillosis and serious infections caused by *Scedosporium* spp., *Fusarium* spp., or invasive fluconazole-resistant *Candida* spp. In one study, voriconazole was more effective than amphotericin B in humans with invasive aspergillosis, regardless of the site of infection, the neutrophil count, and the underlying disease (Herbrecht et al., 2002). Experience with the use of voriconazole in domestic animal species is limited.

Triazoles: Posaconazole and Ravuconazole

Posaconazole and ravuconazole are two second-generation triazoles that will soon be available commercially (Chen and Sobel, 2005). Posaconazole is similar in structure to itraconazole and has potent broad-spectrum antifungal activity. In contrast to voriconazole, posaconazole is active in vitro and in vivo against zygomycetes, a group for which there are

limited options. The drug has a good oral bioavailability and a half-life of 15 h in dogs (Nomeir et al., 2000). Ravuconazole is a derivative of fluconazole with an expanded spectrum against yeast and filamentous fungi. There is no clinical information on the use of these drugs in domestic animal species.

Echinocandins: Caspofungin, Micafungin, and Anidulafungin

Echinocandins are novel lipopeptide antifungal agents which are 1,3- β -D-glucan synthase inhibitors, preventing production of an essential polysaccharide in the cell wall of many fungi. Caspofungin is to date the first echinocandin commercially available. Micafungin and anidulafungin have similar spectra of activity.

Antimicrobial Activity

These drugs are active against yeast of the genus *Candida* including isolates resistant to azoles and amphotericin B. Echinocandins are also highly active against *Aspergillus* spp. These drugs are not active against or have limited activity against *C. neoformans*, *B. dermatitidis*, *C. neoformans*, *C. immitis*, or *Fusarium* spp. Echinocandins possess activity against *Pneumocystis carinii*.

Pharmacokinetic Properties

Echinocandins have limited oral bioavailability and only IV formulations are available (caspofungin) or under development (micafungin, anidulafungin). Caspofungin is mainly eliminated in the urine and the feces as metabolites. No dose adaptation is required in patients with moderate renal insufficiency. Caspofungin has few drug interactions because its metabolism is independent of the cytochrome P₄₅₀ system.

Toxicity and Adverse Effects

Caspofungin is well tolerated. The most common adverse effects are fever, nausea, and phlebitis at the infusion site.

Clinical Applications

A major indication for caspofungin is the treatment of invasive aspergillosis unresponsive to amphotericin B, itraconazole or voriconazole, or in patients intolerant of these drugs. Caspofungin is also similar in efficacy to fluconazole or amphotericin B in the treatment of candidiasis.

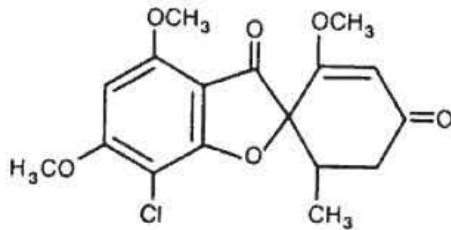


Figure 19.4. Structural formula of griseofulvin.

Other Antifungal Agents for Systemic Use

Griseofulvin

Chemistry

Griseofulvin (Figure 19.4) is a benzofuran cyclohexene antibiotic, a product of *Penicillium griseofulvum*. It is poorly soluble in water.

Mechanism of Action

Griseofulvin is a fungistatic antibiotic that inhibits mitosis, probably by disorganizing the spindle microtubules. It may also interfere with cytoplasmic microtubules.

Antimicrobial Activity

Virtually all dermatophytes of animal origin are inhibited by griseofulvin concentrations of 0.2 to 0.5 $\mu\text{g/ml}$. Other hyphal fungi, yeasts, dimorphic fungi, and bacteria are unaffected. Resistance ($\text{MIC} \geq 3 \mu\text{g/ml}$) to griseofulvin has been described in dermatophytes of human origin.

Pharmacokinetic Properties

Absorption after oral administration depends greatly on particle size. It is enhanced in humans after a high-fat meal. Half-life in humans is about 20 hours but is considerably shorter (less than six hours) in dogs. Most of the drug is excreted in the stool. The drug appears to be metabolized in the liver and increased metabolism may be caused by drugs that induce liver enzymes (e.g., rifampin). It is selectively deposited in the newly formed keratin of hair, nails, and skin, and gradually moves from these deep layers to the site of infection in the superficial keratinized epithelium, where keratinized cells mature and are progressively desquamated. Actively growing fungus may be killed but dormant cells are only inhibited, so that cure occurs when infected keratinized cells are shed. For this reason, treatment is prolonged.

Toxicity and Side Effects

Prolonged medication in humans has occasionally been associated with mild and transient side effects, such as mild central nervous effects (headaches, dizziness, fatigue), photosensitivity, and gastrointestinal disturbances (nausea, vomiting, diarrhea).

Griseofulvin is teratogenic in cats, particularly in the first weeks of gestation. The drug's use results in numerous congenital defects, including brain malformations, skeletal abnormalities, spina bifida, anophthalmia, and atresia ani (Scott et al., 1975). High doses in cats have also been associated with anemia, a possibly idiosyncratic reaction (Kunkle and Meyer, 1987). This may relate to feline immunodeficiency virus infection (Shelton et al., 1990). FIV-positive animals should probably be treated with another drug, such as itraconazole. All cats may exhibit signs of toxicosis including anorexia, vomiting, ataxia, anemia, leukopenia, depression, jaundice, pruritus, and pyrexia (Helton et al., 1986; Wack et al., 1992). These signs are usually, but not always, reversible. Because of the teratogenic effect for all species (Schutte and van den Ingh, 1997), griseofulvin should not be given to any pregnant animal. Dogs and cats may vomit if given griseofulvin on an empty stomach.

Administration and Dosage

The drug should be given for one or two weeks beyond clinical or mycologic cure. A single daily dose of 50 mg/kg can be reduced to 25 mg/kg once clinical response occurs. The optimal dose in cats has not been firmly established but toxicity appears to be idiosyncratic rather than dose-related (Levy, 1991). Griseofulvin is administered orally to ringworm-infected cattle as a 10% mycelial mix, 7.5 to 10 mg/kg for one to three weeks.

Clinical Applications

Griseofulvin is effective only against dermatophytic infections and effective against ringworm only if administered orally. The drug reaches the superficial, dead, parasitized epithelium only through progressive maturation of basal cells. Treatment is thus slow, three to six weeks in dogs and cats. In cattle, probably because of economic considerations, a short treatment of only seven days has been found to render animals free of lesions four weeks or so after treatment. This dosage in larger animals is lower than that used in dogs and cats but is apparently equally effective.

Iodides

Iodides have been used for many years to treat mycotic infections. Their mechanism of action is poorly understood, but action may result from enhancement of the immune response of the host or by spurring the halide-peroxide killing system of phagocytic cells. Amphotericin B and imidazoles also affect the immune system. Historically, sodium iodide has been the treatment of choice in sporotrichosis but itraconazole is becoming the preferred treatment. Ketoconazole and sodium iodide administered together appear to have additive effects. The dose of iodide is 20 mg/kg in cats and 40 mg/kg in dogs. The drug is administered orally once or twice daily and a response occurs in one to four weeks; treatment should be continued for several weeks past clinical cure. Treatment should be temporarily stopped if signs of iodism (e.g., severe coryza, weakness, salivation) occur. Sodium iodide has been used as an adjunct in the treatment of nasal aspergillosis in dogs (Barrett et al., 1977). Sodium iodide has been administered IV, 1 g/15 kg in a 10% solution, in the treatment of ringworm in cattle. The use of iodine preparations in animals that will enter the human food chain is discouraged because of prolonged tissue residues.

Lufenuron

Lufenuron is a benzoylphenyl urea-derived insecticide used as an oral product for flea control in dogs and cats. The drug interferes with chitin synthesis and the deposition of chitin in the cuticle of insects. Chitin is also an important component of the outer cell wall of fungi suggesting that the drug may have antifungal activity as well. In a retrospective study of 297 dogs and cats with dermatophytosis or superficial dermatomycosis, time to resolution of gross lesions was significantly shorter in lufenuron-treated animals than in untreated controls (Ben-Ziony and Arzi, 2000). In contrast, oral lufenuron did not prevent dermatophytosis following experimental infection of cats with *Microsporum canis* (Moriello et al., 2004). There are anecdotal reports of the use of lufenuron for the treatment of fungal endometritis in mares and cutaneous mycosis in chimpanzees (Hess et al., 2002; Dubuis and Lucas, 2003). Lufenuron demonstrates no in vitro activity against *Aspergillus* spp., *Fusarium* spp. and *Coccidioides immitis* (Hector et al., 2005; Scotty et al., 2005). Further therapeutic use of lufenuron as an an-

tifungal agent should be based on proven in vitro activity against specific species of clinically relevant fungi with pharmacokinetic data demonstrating sufficient drug concentration at the site of infection.

Antifungal Drugs for Topical Application

An extensive range of antifungal drugs, some described in Table 19.1, is available for topical application. These preparations include cream, lotion, spray, ointment, powder, solution and nail lacquer for the treatment of onychomycosis. Clotrimazole, itraconazole, miconazole, enilconazole and natamycin are drugs of choice for topical treatment of fungal infections in veterinary medicine. Many other chemicals have antifungal properties, including phenolic antiseptics such as thymol and hexachlorophene; iodides; 8-hydroxyquinoline; quaternary ammonium and bis-quaternary antiseptics; salicylamide; propionic, salicylic, and undecanoic acids; and chlorphenesin. All these compounds and others have been used for the topical treatment of fungal infections of the skin and sometimes of mucosa. The topical antifungal drugs discussed here are of interest for their potency or their broad-spectrum activity.

Natamycin

Natamycin is a fungicidal polyene antibiotic derived from *Streptomyces natalensis* with action against the fungal cell membrane. It is effective against a wide range of filamentous and dimorphic fungi and yeasts (Table 19.4). Natamycin is used for local application against ringworm, in the udder for yeast mastitis, and on the eyes for mycotic keratitis. It is recommended in humans as the initial therapy for fungal keratitis while awaiting identification of the organism and results of susceptibility testing. In terms of in vitro activity against fungal isolates from the eyes of horses with ulcerative keratomycosis, natamycin is equal to miconazole, which is better than itraconazole, which is better than ketoconazole (Brooks et al., 1998). Natamycin is not effective against deep mycotic infections of the eyes because of poor absorption.

Natamycin has been used successfully to treat cows with *Candida* mastitis (20 ml of a 2.5% solution, or 10 ml of a 5% solution, into the affected udder quarter once daily for three days). Total-body spraying or sponging is effective in treatment of ringworm in cat-

Table 19.4. In vitro activity (MIC₉₀, µg/ml) of selected topical antifungal agents against common fungi.

Organisms	Natamycin	Clotrimazole	Nystatin
Filamentous fungi			
<i>Alternaria</i> spp.	2	—	32
<i>Aspergillus fumigatus</i>	8	8	≥64
<i>Fusarium</i> spp.	1	8	≥64
<i>Mucor</i> spp.	1	1	8
<i>Microsporum canis</i>	8	2	4
<i>Trichophyton</i> spp.	8	8	16
Yeasts			
<i>Candida</i> spp.	8	0.5	4
<i>Cryptococcus neoformans</i>	8	4	2
<i>Malassezia pachydermatis</i>	8	2	0.25

tle and horses. It is important that all grooming utensils be thoroughly cleansed or immersed in the natamycin suspension, which should be prepared in plastic or galvanized containers. The drug is used successfully to treat filamentous fungal keratitis in horses, and is the drug of choice for this purpose. A recommended treatment is one drop of a 5% suspension every one or two hours, decreasing to six or eight times daily after a few days. Some clinicians have found natamycin to be locally irritating. Topical application in the treatment of nasal aspergillosis in horses has been clinically effective.

Nystatin

Nystatin is a polyene antibiotic that disorganizes the membrane of fungi, occupying ergosterol-binding sites and altering membrane permeability, so that intracellular ions leak from the cell. The drug is effective against *Candida*, *Malassezia*, *Cryptococcus*, and some dermatophytes. Several *Candida* species other than *C. albicans* are resistant. Nystatin is fungicidal at concentrations about four times MIC. *Prototheca* are reported to be susceptible. Nystatin is used clinically as a topical, broad-spectrum antifungal drug. Clotrimazole has a broader spectrum and is more active. In one study, about one-fifth of yeasts isolated from bovine mastitis were resistant to the drug. A recommended dose is 300,000 units/quarter on three occasions as a single daily dose; the drug can be diluted in saline to 5,000 units/ml and 50 ml administered. Nystatin has been used in dogs to treat *Malassezia* infections of the outer ear and in horses to treat *Candida* metritis.

Azole Antibiotics: Clotrimazole, Enilconazole, Itraconazole, Ketoconazole and Miconazole

Clotrimazole is an azole; its chemical structure and mechanism of action are as described under the systemic azoles. It is inhibitory in vitro to a wide range of filamentous fungi, including *Aspergillus* and dermatophytes, yeasts such as *Candida*, and dimorphic fungi. Concentrations above 10 µg/ml are fungicidal. Few naturally occurring strains of fungi are resistant.

Clotrimazole is a broad-spectrum antifungal agent reserved for topical administration. Local application in mycotic keratitis in horses is well tolerated; the 1% solution is used for *Aspergillus* infections of the cornea. A one-hour application of 100 ml of a 1% solution, administered under general anesthesia, is an effective treatment of nasal aspergillosis in dogs. In one study, a single topical application by either surgically implanted catheters or catheters placed nonsurgically in the nose cured 65% of dogs; a second treatment increased the cure rate to 87% (Mathews et al., 1998). Prolonged recovery in a dog after barbiturate anesthesia and intranasal treatment with clotrimazole for nasal aspergillosis was attributed to hepatic microsomal enzyme induction by clotrimazole (Caulkett et al., 1997).

The drug is used in humans to treat *Candida* vaginitis. In the local treatment of mycotic endometritis in cows or horses, infusions of 400–600 mg clotrimazole every other day for 12 days has been recommended, using sufficient volumes of saline diluent to gently fill the uterus. It may be the drug of choice for yeast mastitis in cows. Intramammary administration of 100–200 mg/quarter/day of 1% solution or cream, on one to four occasions as a single daily dose, has given good clinical results in mycotic mastitis in cows.

Miconazole has similar activity to clotrimazole and has also proven useful for topical treatment of dermatophyte, candidal, *Aspergillus* spp., and *Malassezia* infections.

Intranasal infusion of enilconazole is effective in the treatment of canine nasal aspergillosis both following surgical removal of necrotic and foreign material and on its own, and has become the treatment of choice for nasal aspergillosis in dogs. In one study, rhinoscopic debridement followed by intranasal infusion of 1% or 2% enilconazole was successful in 24 of 26 treated dogs (Zonderland et al., 2002). Itraconazole should be an effective alternative to clotrimazole and enilconazole for local treatment of nasal aspergillosis. Topical

enilconazole has also been used successfully for the treatment of dermatophytosis in small animals.

Enilconazole has been used successfully in environmental decontamination of poultry houses to prevent aspergillosis. Local infusion of enilconazole has been successful in the treatment of guttural pouch mycosis in the horse.

Ketoconazole is also available for topical antifungal therapy, though it is less active in vitro than clotrimazole, itraconazole and miconazole. Like other topical azoles, it is used in the treatment of *Malassezia pachydermatis* ear and skin infections. Miconazole combined with chlorhexidine was more effective as a shampoo than selenium sulfide for treatment of seborrheic dermatitis in dogs caused by *M. pachydermatis* (Bond et al., 1995).

Bibliography

- Andes D. 2004. Clinical utility of antifungal pharmacokinetics and pharmacodynamics. *Curr Opin Infect Dis* 17:533.
- Arceneaux KA et al. 1998. Blastomycosis in dogs: 115 cases (1980-1995). *J Am Vet Med Assoc* 213:658.
- Ball MA, et al. 1997a. Corneal concentrations and preliminary toxicological evaluation of an itraconazole/dimethyl sulphoxide ophthalmic ointment. *J Vet Pharm Ther* 20:100.
- Ball MA, et al. 1997b. Evaluation of itraconazole-dimethyl sulphoxide ointment for treatment of keratomycosis in nine horses. *J Am Vet Med Assoc* 211:199.
- Barrett JP, et al. 2003. A systematic review of the antifungal effectiveness and tolerability of amphotericin B formulations. *Clin Ther* 25:1295.
- Barrett RE, et al. 1977. Treatment and immunological evaluation of three cases of canine aspergillosis. *J Am Anim Hosp Assoc* 13:328.
- Bekerski I, et al. 1999. Safety and toxicokinetics of intravenous liposomal amphotericin B (AmBisome) in beagle dogs. *Pharm Res* 16:1694.
- Bennett JE, et al. 1979. A comparison of amphotericin B alone and combined with flucytosine in the treatment of cryptococcal meningitis. *N Engl J Med* 301:126.
- Bennett JE. 1990. Fluconazole: A novel advance in therapy for systemic fungal infections. *Rev Infect Dis* 12:S263.
- Ben-Ziony Y and Arzi B. 2000. Use of lufenuron for treating fungal infections of dogs and cats: 297 cases (1997-1999). *J Am Vet Med Assoc* 217(10):1510.
- Boothe DM, et al. 1997. Itraconazole disposition after single oral and intravenous and multiple oral dosing in healthy cats. *Am J Vet Res* 58:872.
- Brooks DE, et al. 1998. Antimicrobial susceptibility patterns of fungi isolated from horses with ulcerative keratomycosis. *J Am Vet Med Assoc* 59:138.
- Butler WT, Hill GJ. 1964. Intravenous administration of amphotericin B in the dog. *J Am Vet Med Assoc* 144:399.
- Chen A, Sobel JD. 2005. Emerging azole antifungals. *Expert Opin Emerg Drugs* 10:21.
- CLSI. Reference method for broth dilution antifungal susceptibility testing of yeast. Approved Standard, 2nd ed., M27-A2.
- CLSI. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved Standard, M38-A.
- Cortadellas O. 2003. Initial and long-term efficacy of a lipid emulsion of amphotericin B desoxycholate in the management of canine leishmaniasis. *J Vet Intern Med* 17:808.
- Cuenta-Estrella M. 2004. Combinations of antifungal agents in therapy-what value are they? *J Antimicrob Chemother* 54:854.
- Da Costa PD, et al. 1996. Cataracts in dogs after long-term ketoconazole therapy. *Vet Comp Ophthalmol* 6:176.
- Davis EW, Legendre AM. 1994. Successful treatment of guttural pouch mycosis with itraconazole and topical enilconazole in a horse. *J Vet Intern Med* 8:304.
- Davis JL, et al. 2005. Pharmacokinetics and tissue distribution of itraconazole after oral and intravenous administration to horses. *Am J Vet Res* 66:1694.
- DeKeyser H, Van den Brande M. 1983. Ketoconazole in the treatment of dermatomycosis in cats and dogs. *Vet Quart* 5:142.
- Dubuis E, Lucas D. 2003. Control of cutaneous mycosis in five chimpanzees (*Pan troglodytes*) with lufenuron. *Vet Rec* 152:651.
- Grooters AM, et al. 2003. Update on antifungal therapy. *Vet Clin North Am Small Anim Pract* 33:749.
- Hector RE, et al. 2005. Comparison of susceptibility of fungal isolates to lufenuron and nikkomycin Z alone or in combination with itraconazole. *Am J Vet Res* 66:1090.
- Helton KA, et al. 1986. Griseofulvin toxicity in cats: Literature review and report of seven cases. *J Am Anim Hosp Assoc* 22:453.
- Herbrecht R, et al. 2002. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N Engl J Med* 347:408.
- Hess MB, et al. 2002. Use of lufenuron as a treatment for fungal endometritis in four mares. *J Am Vet Med Assoc* 221:266.
- Hodges RD, et al. 1994. Itraconazole for the treatment of histoplasmosis in cats. *J Vet Intern Med* 8:409.
- Hofbauer B, et al. 2002. In vitro susceptibility of *Microsporum canis* and other dermatophyte isolates from veterinary infections during therapy with terbinafine or griseofulvin. *Med Mycol* 40:179.
- Jacobs GJ, et al. 1997. Cryptococcal infection in cats: Factors influencing treatment outcome, and results of sequential serum antigen titers in 35 cats. *J Vet Intern Med* 11:1.
- Kelly SE, et al. 1995. Long-term survival of four dogs with disseminated *Aspergillus terreus* infection treated with itraconazole. *Aust Vet J* 72:311.
- Korenek NL, et al. 1994. Treatment of mycotic rhinitis with itraconazole in three horses. *J Vet Intern Med* 8:224.
- Kotnik T, et al. 2001. Terbinafine hydrochloride treatment of *Microsporum canis* experimentally-induced ringworm in cats. *Vet Microbiol* 83:161.

- Kotnik T, et al. 2002. Drug efficacy of terbinafine hydrochloride (Lamisil) during oral treatment of cats, experimentally infected with *Microsporum canis*. J Vet Med B Infect Dis Vet Public Health 2002 49:120.
- Krawiec DR, et al. 1996. Use of an amphotericin B lipid complex for treatment of blastomycosis in dogs. J Am Vet Med Assoc 209:2073.
- Kukui H, et al. 2003. Comparison of LNS-AmB, a novel low-dose formulation of amphotericin B with lipid nanosphere (LNS), with commercial lipid-based formulations. Int J Pharm 267:101.
- Kunkle GA, Meyer DJ. 1987. Toxicity of high doses of griseofulvin in cats. J Am Vet Med Assoc 191:322.
- Lamothe J. 2001. Activity of amphotericin B in lipid emulsion in the initial treatment of canine leishmaniasis. J Small Anim Pract 42:170.
- Latimer FG, et al. 2001. Pharmacokinetics of fluconazole following intravenous and oral administration and body fluid concentrations of fluconazole following repeated oral dosing in horses. Am J Vet Res 62:1606.
- Legendre AM, et al. 1984. Treatment of canine blastomycosis with amphotericin B and ketoconazole. J Am Vet Med Assoc 184:1249.
- Legendre AM, et al. 1996. Treatment of blastomycosis with itraconazole in 112 dogs. J Vet Intern Med 10:365.
- Levy JK. 1991. Ataxia in a kitten treated with griseofulvin. J Am Vet Med Assoc 198:105.
- Malik R, et al. 1992. Cryptococcosis in cats: clinical and mycological assessment of 29 cats and evaluation of treatment using orally administered fluconazole. J Med Vet Mycol 30:133.
- Malik R, et al. 1996a. Combination chemotherapy of canine and feline cryptococcosis using subcutaneously administered amphotericin B. Aust Vet J 73:124.
- Malik R, et al. 1996b. Suspected drug eruption in seven dogs during administration of flucytosine. Aust Vet J 74:285.
- Mancianti F, et al. 1998. Efficacy of oral administration of itraconazole to cats with dermatophytosis caused by *Microsporum canis*. J Am Vet Med Assoc 213:993.
- Matot I, Pizov R. 2000. Pulmonary extraction and accumulation of lipid formulations of amphotericin B. Crit Care Med 28:2528.
- McDonald JS, et al. 1980. In vitro antimycotic sensitivity of yeasts isolated from infected bovine mammary glands. Am J Vet Res 41:1987.
- McMullan WC, et al. 1977. Amphotericin B for the treatment of localized subcutaneous phycomycoses in the horse. J Am Vet Med Assoc 170:1293.
- Medleau L, et al. 1990. Evaluation of ketoconazole and itraconazole for treatment of disseminated cryptococcosis in cats. Am J Vet Res 51:1454.
- Medleau L, et al. 1995. Itraconazole for the treatment of cryptococcosis in cats. J Vet Intern Med 9:39.
- Medleau L, Chalmers SA. 1992. Ketoconazole for treatment of dermatophytosis in cats. J Am Vet Med Assoc 200:77.
- Moore R. 1982. Treatment of feline nasal cryptococcosis with 5-flucytosine. J Am Vet Med Assoc. 15:181.
- Moriello KA, et al. 2004. Efficacy of pre-treatment with lufenuron for the prevention of *Microsporum canis* infection in a feline direct topical challenge model. Vet Dermatol 15:357.
- Moriello KA. 1986. Ketoconazole: clinical pharmacology and therapeutic recommendations. J Am Vet Med Assoc 188:303.
- Moriello KA. 2004. Treatment of dermatophytosis in dogs and cats: review of published studies. Vet Dermatol 15:99.
- Moriello KA, DeBoer DJ. 1995. Efficacy of griseofulvin and itraconazole in the treatment of experimentally induced dermatophytosis in cats. J Am Vet Med Assoc 207:439.
- Nomeir AA, et al. 2000. Pharmacokinetics of SCH 56592, a new azole broad-spectrum antifungal agent, in mice, rats, rabbits, dogs, and cynomolgus monkeys. Antimicrob Agents Chemother 44:727.
- Orosz SE, et al. 1996. Pharmacokinetic properties of itraconazole in Blue-fronted Amazon parrots (*Amazona aestiva aestiva*). J Avian Med Surg 10:168.
- Plotnick AN, et al. 1997. Primary cutaneous coccidioidomycosis and subsequent drug eruption to itraconazole in a dog. J Am Anim Hosp Assoc 33:139.
- Prades M, et al. 1989. Body fluid and endometrial concentrations of ketoconazole in mares after intravenous injection or repeated gavage. Equine Vet J 21:211.
- Rex JF, Pfaller MA. 2002. Has antifungal susceptibility testing come of age? Clin Infect Dis 35:982.
- Rex JH, et al. 2003. A randomized and blinded multicenter trial of high-dose fluconazole plus placebo versus fluconazole plus amphotericin B as therapy for candidemia and its consequences in nonneutropenic subjects. Clin Infect Dis 36:1221.
- Richardson RC, et al. 1983. Treatment of systemic mycoses in dogs. J Am Vet Med Assoc 183:335.
- Roffey SJ, et al. 2003. The disposition of voriconazole in mouse, rat, rabbit, guinea pig, dog, and human. Drug Metab Dispos 31:731.
- Rosales MS, et al. 2005. Comparison of the clinical efficacy of oral terbinafine and ketoconazole combined with cephalixin in the treatment of *Malassezia* dermatitis in dogs—a pilot study. Vet Dermatol 16:171.
- Rubin SI, et al. 1989. Nephrotoxicity of amphotericin B in dogs: A comparison of two methods of administration. Can J Vet Res 53:23.
- Schutte JG, van de Ingh TSGM. 1997. Microphthalmia, brachygnathia superior, and palatocheiloschisis in a foal associated with griseofulvin administration to the mare during early pregnancy. Vet Quart 19:58.
- Scott FW, et al. 1975. Teratogenesis in cats associated with griseofulvin therapy. Teratology 11:79.
- Scotty NC, et al. 2005. In vitro efficacy of lufenuron against filamentous fungi and blood concentrations after PO administration in horses. J Vet Intern Med 19:878.
- Sharkey PK, et al. 1991. High-dose itraconazole in the treatment of severe mycoses. Antimicrob Agents Chemother 35:707.
- Sharp NJH, et al. 1991. Treatment of canine nasal aspergillosis/penicilliosis with fluconazole (UK-49,858). J Small Anim Pract 32:513.
- Sharp NJH, Sullivan M. 1989. Use of ketoconazole in the treatment of canine nasal aspergillosis. J Am Vet Med Assoc 194:782.

- Shaw SE. 1988. Successful treatment of 11 cases of feline cryptococcosis. *Aust Vet Pract* 18:135.
- Shelton GH, et al. 1990. Severe neutropenia associated with griseofulvin therapy in cats with feline immunodeficiency virus. *J Vet Intern Med* 4:317.
- Tiches D, et al. 1998. A case of canine central nervous system cryptococcosis: management with fluconazole. *J Am Anim Hosp Assoc* 34:145.
- Vaden SL, et al. 1997. Fluconazole in cats: Pharmacokinetics following intravenous and oral administration and penetration into cerebrospinal fluid, aqueous humour and pulmonary epithelial lining fluid. *J Vet Pharm Ther* 20:181.
- Verweij PE, et al. 1997. High-dose itraconazole for the treatment of cerebral aspergillosis. *Clin Infect Dis* 23:1196.
- Wack RF, et al. 1992. Griseofulvin toxicity in four cheetahs (*Acinonyx jubatus*). *J Zoo Wild Med* 23:442.
- Wilkinson GT, et al. 1983. Successful treatment of four cases of feline cryptococcosis. *J Small Anim Pract* 24:507.
- Willard MD, et al. 1986a. Ketoconazole-induced changes in selected canine hormone concentrations. *Am J Vet Res* 47:2504.
- Willard MD, et al. 1986b. Effect of long-term administration of ketoconazole in cats. *Am J Vet Res* 47:2510.

Antiviral Chemotherapy

Dongwan Yoo

Viruses are the simplest of organisms, with genome sizes of only 1.5 to 200 kilobases of DNA or RNA. Because of the limited capacity of their genomes, viruses do not carry all the genes required for multiplication, but rather carry only the most essential genes. Viruses pirate the cellular machinery of the infected cell for multiplication. Therefore, virus multiplication is strictly dependent on the metabolic pathways of the host cell.

Despite the simple nature of viruses, development of antiviral agents has been very slow, the major reason being the inability to distinguish viral-specific multiplication pathways from normal cellular metabolic processes. Since the inhibitory effect of an antiviral agent may be general for both viral and cellular processes, efficacy demonstrated *in vitro* is far remote from clinical efficacy, mainly because of the toxic effects of the antiviral agents. In the past 2 decades, however, improved understanding of the replication cycles of viruses has led to the development of selective antiviral drugs to inhibit specific stages of virus replication.

A general overview of the life cycle of viruses reveals some stages that may be useful to target for chemotherapeutic inhibition of virus replication (Figure 20.1).

Virus replication starts with attachment of the virus to specific receptors on the cell surface, followed by penetration into the cell either by direct fusion of the viral membrane with the plasma membrane or by endocytosis, depending on the particular virus. The attachment and penetration steps include specific interactions between viral proteins and cellular proteins, specific interactions which can be targeted for antiviral inhibitors. Once internalized into the cell, the viral genome is released for synthesis of viral proteins and for replication of viral DNA or RNA. Replication of

viral genomes is generally mediated by virally-encoded enzymes, either by DNA or RNA polymerases or by nucleoside kinases, processes which can also be a target for specific inhibition of the virus. Many of the licensed drugs currently used in antiviral chemotherapy are directed against the synthesis of viral DNA or RNA. When viral messenger RNAs (mRNA) are synthesized, translation of the viral mRNAs can be a target to block virus multiplication. Translation of mRNAs can be blocked either in a general fashion to prevent both viral and cellular mRNAs, or in a rather selective way to prevent only the viral specific mRNA using a specific antisense oligonucleotide. Once mRNAs are translated, the proteins are often processed further to yield smaller subunit proteins by specific proteolytic cleavages or to add carbohydrates, lipids, or phosphate groups to the protein. Inhibition of proteolytic cleavage or of the addition of carbohydrates has also been a target for antiviral therapy. Specific inhibition of protease activity is an attractive target, and antiprotease drugs have been recently licensed to treat infections caused by the human immunodeficiency virus (HIV).

This chapter describes basic strategies for developing antiviral drugs, the modes of action of some of the successful drugs, and their veterinary applications.

Inhibitors of Virus Attachment to Cells

Immunoglobulin

Administration of immunoglobulin can block the initial stage of the virus life cycle by preventing the attachment of the virus to cells. Immunoglobulins are prepared from pooled plasma to contain predominantly IgG selected for high titers of antibody (hyper-

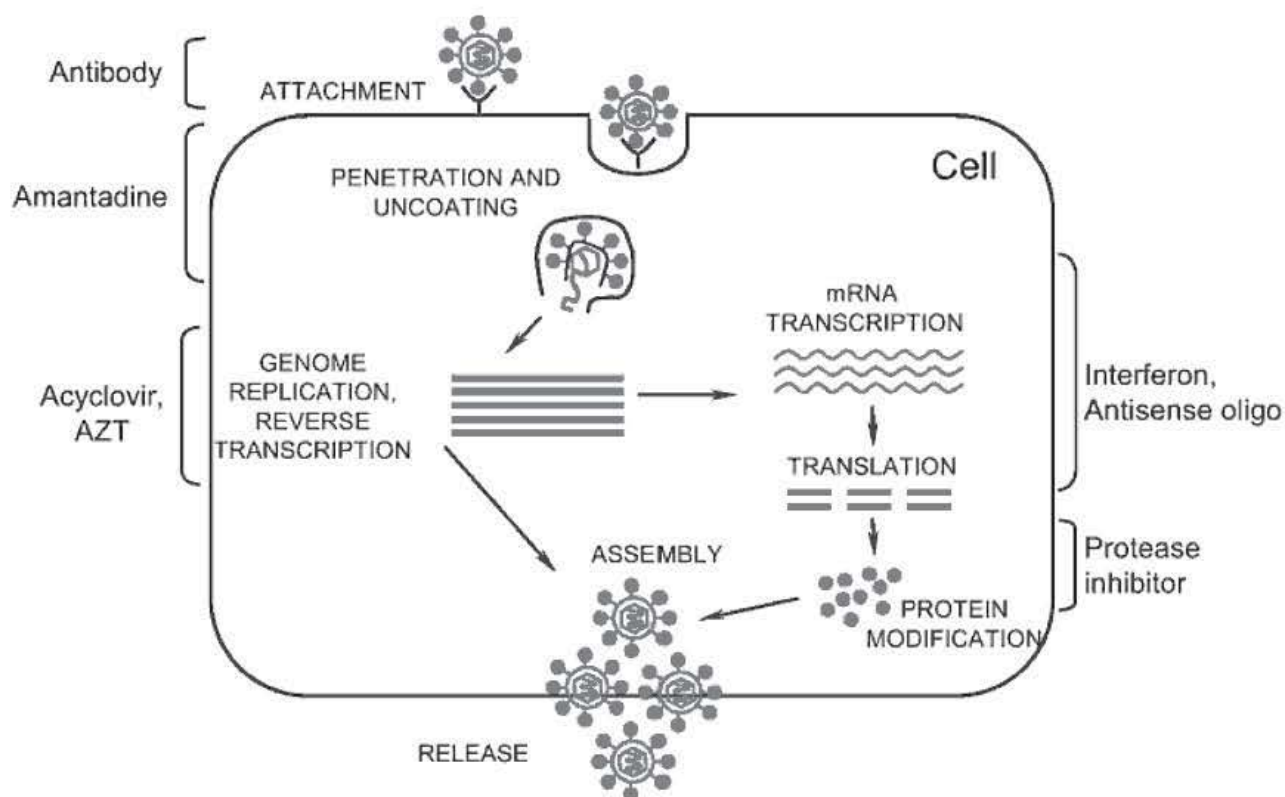


Figure 20.1. Viral replication process within a host cell, showing targets for antiviral drugs.

immune) for specific viruses. Immunoglobulins are more effective when used for prophylactic purposes rather than therapeutic purposes, because the passively administered immunoglobulins are present in the extracellular phase where the antibodies can neutralize viruses before they enter into cells. In veterinary medicine, oral administration of colostrum containing high titers of specific antibody has been used to prevent transmissible gastroenteritis virus infection in swine. A similar approach has been practiced in newborn calves to prevent neonatal scours caused by bovine rotavirus and bovine coronavirus. Vaccination of dams with appropriate antigens increases the specific antibody titers in the colostrum, and the suckling offspring is protected from enteric infections during the nursing period (Lee et al., 1995). In human medicine, immunoglobulins are available predominantly for intramuscular (IM) administration. Since large volumes of immunoglobulin cannot be administered IM, the amount which can be given by this route is limited. It is not possible to administer older immunoglobulin preparations IV because of the tendency for IgG to form aggregates and to fix comple-

ment in the body. Recent advances in cold fractionation procedures have made it possible to prevent IgG aggregation, and newer preparations are available for IV administration (Pennington, 1990). Immunoglobulins are safe with very few side effects. In humans, immunoglobulins are used for pre-exposure prevention of hepatitis A and for post-exposure prevention of hepatitis B and rabies virus infections. Intravenous administration of immunoglobulin is useful to treat the immunodeficiency state. In AIDS patients, the immunoglobulin itself does not contain antibodies for HIV, but rather contains antibodies to other infectious agents, which function to prevent opportunistic infections during HIV immunosuppression. Recent studies in laboratory animals and anecdotal evidence in people suggest that human intravenous immunoglobulin has prophylactic and therapeutic efficacy against infection with West Nile virus (Ben-Nathan et al., 2003; Julander et al., 2005).

Receptor Homologs

A number of compounds inhibit initial attachment of virus to cell receptors. Among the most extensively

studied is the WIN compound for picornavirus (Rueckert, 1996). This drug inserts into a hydrophobic pocket within the canyon floor on the surface of the virus particle. Binding of the drug triggers deformation of the receptor binding site of the virus, leading to inhibition of the virus attachment to cellular receptor. Since many serotypes of human rhinovirus utilize a single cellular receptor (ICAM-1), the drug is effective against most rhinoviruses. Rhinoviruses are the major cause of the common cold, and the drug is most effective when delivered intranasally.

Inhibitors of Virus Uncoating

Amantadine and Rimantadine (Ion Channel Blocking Agents)

Amantadine and its structural analog, rimantadine, were first recognized to possess antiviral activity for influenza A virus in cell culture and in mouse and ferret models in the 1960s. The spectrum of these drugs was limited to type A influenza virus only, with no activity for type B influenza virus, parainfluenza virus, and other respiratory viruses. Only two decades later was the molecular basis for the inhibitory mechanisms precisely elucidated. Influenza virus contains single stranded RNA as a genome consisting of eight RNA segments. Individual RNA segments are packaged into viral nucleoprotein complex (vRNP) in association with M1 protein and the nucleocapsid protein (NP). Following attachment to the cellular receptor to initiate infection, influenza virus is internalized by receptor-mediated endocytosis and is delivered to endosomes. The M2 protein, a small protein associated with the membrane of the virus, appears to function as an ion channel for proton influx from the endosome into the interior of the virion before viral membrane and endosomal membrane fusion occurs. Low pH in the virion causes dissociation of the M1 protein from the RNP complex. In parallel, low pH in the endosome triggers fusion of viral membrane with the endosomal membrane. This fusion allows entry of the viral RNP complex and the M1 protein into the cytoplasm, where the vRNP is further transported to the nucleus to initiate replication and transcription of viral RNA. The M2 protein is the target of amantadine, which binds to the protein and blocks its ion channel activity. As a result, the pH in the virion is unchanged and the M1 protein is therefore not dissoci-

ated from the vRNP complex. Association of the M1 protein with the RNP complex prevents transport of the RNP to the nucleus, thereby inhibiting the subsequent replication transcription of the viral genome. Amantadine is highly specific for influenza virus type A. The sequence of the M2 protein is conserved in the transmembrane domain for both human and avian strains of influenza A virus, and thus the drug is effective for both strains of virus.

Clinical Application

Both amantadine and rimantadine are available in oral formulations. The drugs are absorbed well in the body with peak plasma concentration occurring 2-4 hours after oral administration. Amantadine is metabolically stable, and therapeutic concentrations can be achieved in the lungs, nasal mucus, and saliva. Protection efficacy against the influenza A virus infection is up to 90% in humans. The disposition of amantadine has been studied in horses. Intravenous injection of amantadine resulted in therapeutic drug concentrations, but dose-dependent CNS signs were observed in some horses (Rees et al., 1997). However, commercial preparations are available only in tablet form or syrup, and oral administration of amantadine to horses results in low and variable plasma concentrations. In contrast, prophylactic oral administration of rimantadine to horses at 30 mg/kg of body weight twice daily appeared safe, resulted in therapeutic serum concentrations, and minimized the clinical signs following experimental challenge with influenza (Rees et al., 1999). The drug cost is prohibitive in most mature horses (approximately \$600 per day).

Inhibitors of DNA or RNA Synthesis

Nucleoside Analogs

Idoxuridine, Vidarabine, Trifluridine

Antiviral drugs used in the early years were mostly nucleoside analogs synthesized as anticancer drugs. Since smallpox and herpesvirus infections were among the most important viral diseases during that time, the anticancer drugs were tested for antiviral activities for these viruses. Idoxuridine, vidarabine, and trifluridine are potent inhibitors of herpesvirus DNA synthesis. During synthesis of the DNA strand, these drugs are incorporated into the growing chain of viral DNA, resulting in the misinterpretation of the genetic codes.

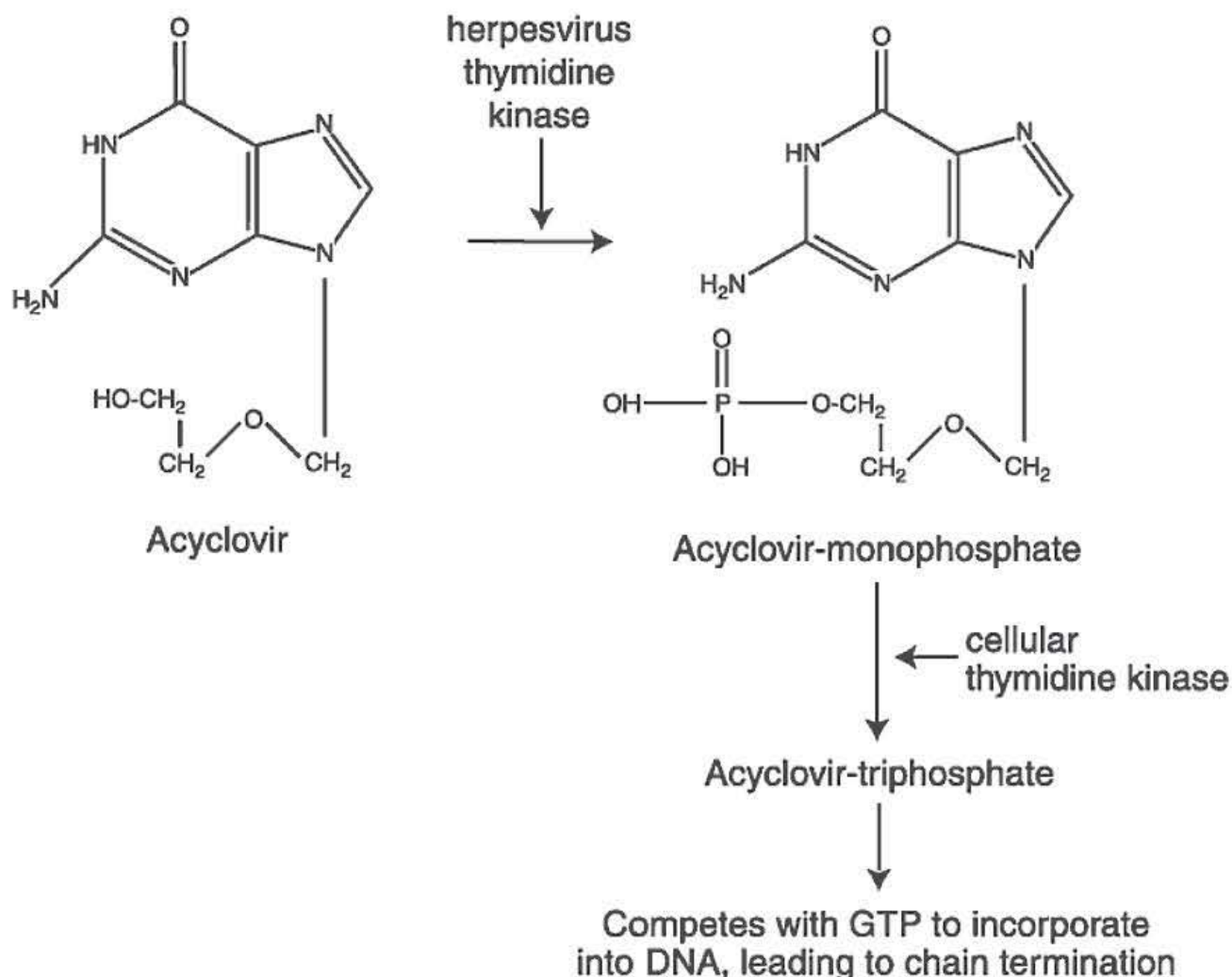


Figure 20.2. Metabolism of acyclovir in the cell to produce the active antiviral drug.

However, the blocking effects are not restricted to the virus alone, but are also directed toward the normal cells resulting in significant cytotoxicity, such as loss of hair, anemia, and neutropenia. Therefore, these drugs are recommended for use as topical applications.

Acyclovir and Related Drugs

The discovery of acyclovir was a major breakthrough in antiviral chemotherapy. Acyclovir appears to be selective for viral rather than normal cellular functions. This specific selectivity results in fewer cytotoxic effects. The molecular target for acyclovir is the viral DNA polymerase. Acyclovir has potent activity against herpesviruses, but not against other viral families. In herpesvirus infected cells, acyclovir is phosphorylated by thymidine kinase to become acycloguanosine

monophosphate (ACG-P). The thymidine kinase encoded by herpesviruses has broader specificity than cellular kinase, and thus acycloguanosine is selectively kinased by the herpesvirus thymidine kinase (Fig 20.2).

ACG-P is then further phosphorylated to acycloguanosine triphosphate (ACG-PPP) by a normal cellular kinase. ACG-PPP competes with guanosine triphosphate (GTP) for incorporation into a DNA chain. Since acyclovir lacks the 3' hydroxyl group, once ACG-PPP is incorporated into the growing DNA strand, further elongation of the DNA chain is terminated. Acyclovir is not phosphorylated in normal cells because of the lack of herpesvirus encoded thymidine kinase, and thus acyclovir is essentially nontoxic to uninfected cells. In humans, acyclovir and its derivatives are drugs of choice for treating the herpes simplex

virus types 1 (HSV-1) and 2 (HSV-2) and varicella-zoster virus (VZV, chicken pox) infections.

Ganciclovir is a guanosine analog structurally similar to acyclovir, but approximately 100 times more potent against cytomegaloherpervirus (CMV) than acyclovir. The reason for its greater activity against CMV is not clear. Since more than 90% of the administered acyclovir is recovered intact in urine, several prodrugs have been developed to increase the bioavailability. Valaciclovir is a valyl ester of acyclovir and is an oral prodrug that is rapidly converted to acyclovir and L-valine, the essential amino acid, by intestinal and hepatic metabolism. Therefore, valaciclovir is similar to acyclovir but has greater bioavailability. Penciclovir is a synthetic acyclic guanine derivative, chemically similar to ganciclovir but functionally closer to acyclovir. Bioavailability is poor for penciclovir, and an orally administered prodrug has been developed. Famciclovir is a prodrug of penciclovir with a higher bioavailability and elimination rate. The members of this series of acyclovir derivatives are all effective for HSV-1, HSV-2, and VZV, with a relatively high elimination rate of more than 90% through urine.

Ribavirin

When ribavirin was first synthesized, it was thought to be a promising antiviral agent since the drug showed a wide spectrum of antiviral activity for both DNA and RNA viruses in cell culture and in experimental animals. Ribavirin resembles guanosine in its structure but the mode of action is not precisely defined. It appears that ribavirin functions at multiple stages of cellular process. In cells, ribavirin is phosphorylated by cellular kinase and converted to ribavirin monophosphate. Ribavirin monophosphate inhibits inosine monophosphate dehydrogenase, an enzyme essential for the synthesis of GTP. GTP is an essential component for DNA synthesis, and its inhibition results in the decrease of the cellular pool of GTP, preventing DNA synthesis. Ribavirin also inhibits the capping process of the 5' end of mRNA. In eukaryotic cells, 5' capping of mRNA mediates translation initiation, and thus its inhibition blocks protein synthesis. Ribavirin also directly inhibits the RNA-dependent RNA polymerase of influenza virus.

Clinical Application of Nucleoside Analogs

Idoxuridine and trifluridine have been successfully used topically in horses with herpesvirus keratopathy.

Foals suffering from equine herpesvirus type 2 (EHV-2) keratopathy and conjunctivitis with nodular corneal opacity infection were successfully treated with ophthalmic medication containing 0.5% idoxuridine during an outbreak on a large breeding farm (Collinson et al., 1994). Trifluridine and idoxuridine have also been tested for feline herpesvirus type 1 infection (FHV-1), which causes acute respiratory disease, keratitis, and conjunctivitis in cats. Both trifluridine and idoxuridine as 0.1% ophthalmic solution are effective for FHV-1 conjunctivitis. Trifluridine has better corneal penetration than idoxuridine. Trifluridine eye drops may be applied 6 times daily for 2 to 3 weeks. Idoxuridine was ineffective for treatment of systemic canine herpesvirus (CHV) infection. In contrast, pups given vidarabine survived CHV infection.

A major toxic effect of ribavirin is anemia. The phosphorylated ribavirin diffuses into and accumulates in erythrocytes without further processing. The erythrocytes become damaged and are subsequently eliminated from the circulation, causing anemia. Although the adverse effect is reversible, the half-life of ribavirin in erythrocytes is 40 days in humans, and thus the adverse effect is prolonged. In infants with respiratory syncytial virus, ribavirin reduces the severity and duration of illness. Ribavirin has been shown to possess therapeutic effects for bunyaviruses, especially human hantavirus infection, arenaviruses, and reoviruses, especially when given at an early stage of illness. Ribavirin is an expensive drug and the drug cost may be more than \$400 per day.

Ribavirin has been shown to be effective against feline infectious peritonitis virus (FIPV) in vitro and in vivo. Ribavirin treatment of cats infected with FIPV, combined with human interferon alpha (IFN- α) increases survival time and reduces clinical signs of the disease. While interferon is well tolerated in cats, ribavirin is toxic at a dosage of 10 mg/kg. A lower dosage of ribavirin (5 mg/kg), combined with interferon is recommended for FIPV infection. Ribavirin treatment did not modify the clinical progression of feline calicivirus (FeCV) infection in cats, nor did it reduce the duration of virus shedding even when therapy was initiated within a day of exposure. Toxic effects of ribavirin in cats may include anemia, leukopenia, and thrombocytopenia (Povey, 1978). In ferrets, ribavirin has significant therapeutic effects on influenza A virus infection.

Acyclovir as a 3% ointment is used to treat chronic

FHV-1 conjunctivitis, although results are variable (Greene, 1998). The ointment may cause irritation when applied to the eyes of cats. Acyclovir has been tested for systemic treatment of FHV-1 infection but its efficacy is far less (80 times) in cats than in humans. Cats treated with valaciclovir for FHV-1 infection became more lethargic and dehydrated. Total WBC and neutrophil counts were significantly decreased, and even high doses did not suppress FHV-1 replication in acutely infected cats (Nasisse et al., 1987). Valciclovir was ineffective against systemic FHV-1 infection in cats, and moreover, cats are very sensitive to its toxic effects.

Acyclovir is active against equine herpesvirus type 1 (EHV-1) *in vitro*. There are anecdotal reports of the use of oral acyclovir to treat foals or adult horses with EHV-1 infections. However, acyclovir is poorly absorbed in horses and oral administration does not result in therapeutic drug concentrations (Wilkins et al., 2005). Acyclovir suspension for IV use is cost prohibitive for most equine patients. Acyclovir is also active *in vitro* for other herpesviruses such as turkey herpesvirus and Marek's disease virus (MDV). Acyclovir suppresses the development of tumors in birds infected with MDV (Samorek-Salamonowicz et al., 1987).

Pyrophosphate Analogs

Foscarnet is a pyrophosphate analog which directly inhibits both DNA polymerase and RNA polymerase. Foscarnet is chemically distinct from nucleoside analogs and does not incorporate into the DNA or RNA chain but inhibits the synthesis of nucleic acids. The oral bioavailability of foscarnet is only 10% in dogs and 35% in cats, and thus the drug must be given by IV injection. Although foscarnet is rapidly eliminated from plasma in young cats, clearance is slower in older cats, and approximately 5-10% of the administered foscarnet accumulates in bones (Swenson et al., 1990). Foscarnet is strongly nephrotoxic and renal dysfunction is a common complication.

Inhibitors of Reverse Transcription

The RNA genome of retroviruses requires conversion to DNA to proceed to further replication. Retroviruses encode reverse transcriptase for this process. Since this process is unique and essential for retrovirus replica-

tion, it is the major target for antiviral chemotherapy. The first anti-retroviral compound licensed for human use was a thymidine analog, zidovudine (AZT, azidothymidine). In virus-infected cells, AZT is phosphorylated by cellular kinases and is subsequently converted to AZT triphosphate (AZT-PPP) (Figure 20.3).

AZT-PPP then competes with thymidine triphosphate (TTP) for the synthesis of the DNA strand. However, the affinity of AZT-PPP is 100 times its affinity for the cellular DNA polymerase. Thus, AZT-PPP is preferentially incorporated into the growing chain of viral DNA by the reverse transcriptase, leading to the premature termination of viral DNA. It is important to note that since the retrovirus genome is integrated into the host chromosome indefinitely, AZT will only suppress active virus replication. Therefore, in individuals already infected, it is not possible to eliminate the integrated viral genome from the body with chemotherapeutic agents. Several other nucleoside analogs have been licensed for treating HIV in humans: dideoxyinosine (ddI, didanosine), dideoxycytidine (ddC, zalcitabine), d4T (stavudine), and 3TC (lamivudine). These analogs are all chain terminators that inhibit viral DNA synthesis.

Zidovudine has been used to treat cats affected with feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV). Pre-treatment with up to 50 mg/kg of zidovudine did not prevent cats from developing viremia and lymphadenopathy following infection, although the onset of immunodeficiency was delayed at the highest dose (Smyth et al., 1994a). High doses of zidovudine cause severe anemia. Zidovudine was therefore much less effective in cats than expected from the results of *in vitro* studies. Other nucleoside analogs including ddI, ddC, and ddA have been shown to have antiviral activity against FIV in cell culture (Smyth et al., 1994b), but their clinical effects are unknown. For FeLV, the time of administration after infection is crucial for therapeutic effects. When administered immediately following infection, AZT abrogates virus replication at 10 mg/kg. However, if therapy is initiated 4 weeks after infection when cats have already developed viremia, AZT therapy does not prevent progression of infection even at high doses (Tavares et al., 1987). The efficacy of stampidine, a novel nucleoside reverse transcriptase inhibitor, was recently studied in cats chronically infected with FIV. A 4-week stampidine treatment course with twice-daily administration was well tolerated by cats at cu-

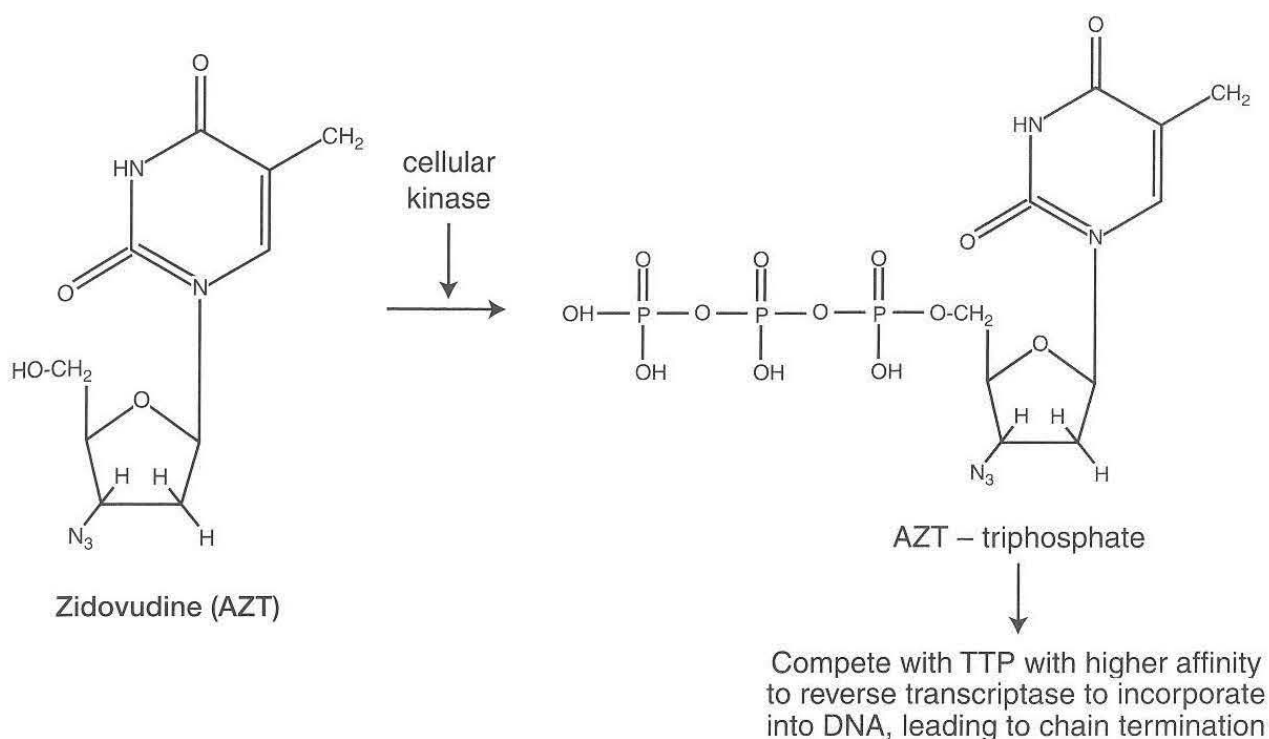


Figure 20.3. Metabolism of zidovudine in the cell to produce the active drug.

mulative dose levels as high as 8.4 g/kg and exhibited a dose-dependent antiretroviral effect. Most treated cats showed a therapeutic response, as evidenced by a >1-log reduction in the FIV load in peripheral blood mononuclear cells within 2 weeks of initiating therapy (Uckun et al., 2003).

Inhibitors of mRNA Translation

Antisense Oligonucleotide as Selective Inhibitors

The sequence of a nucleotide chain that contains the information for protein synthesis is called the sense sequence. The nucleotide strand that is complementary to the sense sequence is its antisense sequence. Antisense drugs recognize and bind to the nucleotide sense sequence of specific mRNA molecules, thereby preventing the synthesis of specific proteins and leading to destruction of the mRNA molecules by ribonucleases in the cell. Antisense approaches have been used commercially to interfere with expression of plant genes, such as in genetically engineered tomatoes to interfere with fruit softening during the ripening process. The first antisense-based antiviral agent ap-

proved in the United States was fomivirsen (Fox, 1998). Fomivirsen is licenced to treat retinitis caused by cytomegalovirus infection in patients with AIDS. Fomivirsen is an antisense oligonucleotide, binding to the complementary strand of the cytomegalovirus gene and blocking the regular function of the viral messenger RNA to synthesize the protein (Figure 20.4). The product is administered locally into the eye by intravitreal injection. This drug appears to be safe and effective with minimal side effects since it is highly selective for viral mRNA. Fomivirsen is significantly more effective in CMV retinitis than the conventional antiviral drugs, ganciclovir and foscarnet.

Interferons

Interferons (IFNs) are cytokines, a large family of cellular proteins that regulate immune responses by coordinating the activities of various types of immune cells. IFNs play a major role in host defenses against viral infection. Human IFNs are divided into type 1 (IFN- α and IFN- β) and type 2 (IFN- γ). IFN- α and IFN- β are produced from leukocytes and fibroblasts, respectively, in response to virus infections, especially by RNA viruses, whereas IFN- γ is released from T-

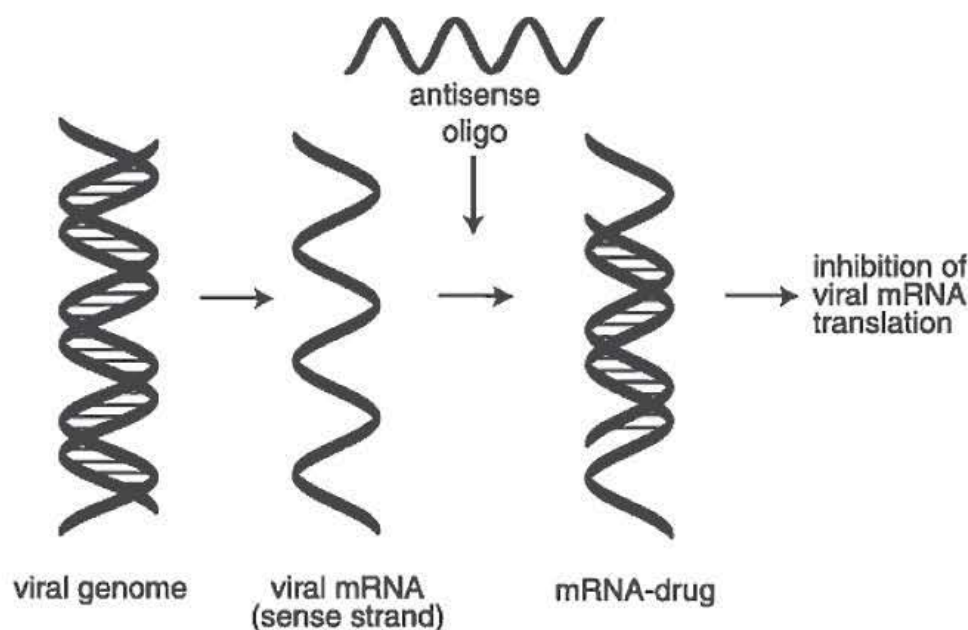


Figure 20.4. Mode of action of antisense-based antiviral drugs.

lymphocytes by antigen or mitogen stimulation. Based on its cellular origin, IFN- γ is classified as a lymphokine. The principal activity for IFN- α and - β is antiviral action while IFN- γ possesses immunomodulatory and antiproliferative effects (Vilc k and Sen, 1996). IFN- α and IFN- β share common receptors on their target cells and are rapidly internalized after binding to cell membrane receptors. IFNs increase antibody production and natural killer cell activity, as well as the expression of class I major histocompatibility complex antigen on the cell surface, thus enhancing recognition of virally infected cells by the immune system. The antiviral activity of these IFNs is rather indirect, altering host cell metabolism to impair protein synthesis and thereby suppressing assembly of viral components. The antiviral effects of IFNs result from induction of several proteins in the exposed cells. These proteins are involved in protecting cells from a range of viral infections. For example, 2', 5'-oligoadenylate synthetase causes breakdown of mRNAs, and the protein kinase activated by double-stranded RNA blocks protein translation.

IFN- γ has distinct immunomodulating effects, inducing expression of class II major histocompatibility complex antigen on macrophages (Vilc k and Sen, 1996). IFN- γ has a central role in activating macrophages and thus has considerable potential clinical use in enhancing resistance to intracellular pathogens. Its

use for this purpose against specific infections is under active investigation, inspired in part by the variety of opportunistic intracellular infections in human patients with AIDS.

IFN- α and IFN- β are active against a broad range of viruses while IFN- γ has additional activity against intracellular pathogens, as described. The immunomodulatory effects of IFN- γ may have clinical effects that cannot be predicted by current theoretical considerations; thus experimental and clinical studies are required before IFN- γ can be recommended for specific purposes.

Clinical Applications

Recombinant DNA technology has resulted in the production of large quantities of human, bovine, and feline IFNs for assessment of their clinical potential. Other species-specific IFNs may also be produced. Although IFNs are produced in most animal species, they (especially IFN- β and IFN- γ) tend to be active mainly in the species in which they are induced. However, they possess no viral specificity, so that the interferon produced in response to one virus is active against other viruses. The clinical role of IFNs in prophylaxis and treatment of viral infections is of considerable interest in human and veterinary medicine.

IFN- α can be administered SC, IM, IV, or locally. IFNs are generally administered IM and have serum

half-lives of 3–8 hours. Continued use of human IFNs in animals is eventually accompanied by development of neutralizing antibodies (Zeidner et al., 1990). Synergism with other antiviral drugs has sometimes been described (Weiss, 1989). Synergism of IFN- γ with azithromycin against experimental toxoplasmosis was described (Aranjo and Remington, 1991).

Among potential uses of IFNs in human medicine are intranasal application of IFN- α in prevention of rhinovirus infections, an effect also described in cattle (Rosenquist and Allen, 1990), the treatment of chronic viral hepatitis, the intralesional treatment of some papillomavirus infections, and the treatment of herpes simplex infections. The suggestion is currently being explored that IFN might have an adjunctive role in managing certain neoplasms and might provide effective therapy in rabies and hemorrhagic fevers. The adverse effects of IFNs suggest that these drugs will generally be reserved for severe infections in humans and animals, or for local use.

In cattle, IM or intranasal treatment of calves with human IFN- α reduced morbidity caused by bovine herpesvirus 1 and *Mannheimia haemolytica*, possibly due to the immunomodulatory activity of the drug (Babiuk et al., 1987). No antiviral effect was observed in calves infected with respiratory syncytial virus (Dennis et al., 1991). Calves treated with bovine IFN- α were protected against experimental vaccinia infection (Schwerset al., 1989). Recombinant bovine IFN- γ administered by the intramammary route considerably reduced the severity of experimentally induced acute *E. coli* mastitis in cattle (Sordillo and Babiuk, 1991) and enhanced mammary phagocyte function (Fox et al., 1990). In cats, oral dosage with bovine IFN- β or human IFN- α resulted in favorable resolution of nonregenerative anemia after feline leukemia virus infection. Treatment with high doses of human IFN- α temporarily suppressed clinical signs of disease and increased survival time in experimentally induced feline infectious peritonitis (Weiss et al., 1990). Feline IFN was especially effective in reducing clinical signs caused by FHV and FCV in cats when administered at 5×10^6 units/kg. In a study involving clinically ill cats infected with FeLV, therapy with *Staphylococcus* protein A and oral interferon- α resulted in improved health status over the 10-week treatment period (McCaw et al., 2001). In a recent study, recombinant feline IFN- ω initially had significant therapeutic effects on clinical signs and later on survival of cats

with FeLV infection and FeLV/FIV coinfection (de Mari et al., 2004). Recent research in laboratory animals and anecdotal evidence in people suggest that IFN- α is effective in the treatment of West Nile virus infections (Morrey et al., 2004).

Persistent fatigue and acute flu-like illness (fever, chills, myalgia) always accompany IFN therapy in humans, although this may decline with continued administration of IFN- α and - β , but not - γ . Peripheral neuropathy and neuropsychiatric symptoms may also occur. Mild, reversible neutropenia is common. Fever and neutropenia were reported in calves treated with IFN- α (Dennis et al., 1991). Transient anorexia and weight loss have been described in cats (Zeidner et al., 1990). Adverse effects are dose-related and may be substantial.

Inhibitors of Post-translation Modification

Proteolytic Cleavage Inhibitors

Many viral proteins undergo further processing after being translated, and cleavage of the precursor proteins is required to activate enzymatic function, fusion, and maturation of the virion. During the replication of retrovirus, the viral genome is translated to produce two major polypeptides, *gag* and *gag-pol*. A portion of the *gag-pol* polypeptide is a virus-coded protease enzyme, and by this enzyme activity, the *gag* and *gag-pol* polypeptides are self-cleaved to yield various functional proteins essential for the replication cycle. Inhibition of the protease enzyme can block cleavage of the viral polypeptide, preventing virus replication. In HIV, the viral protease is a member of the aspartic protease family. The HIV protease is structurally different from mammalian aspartic proteases such as renin, pepsin, and gastricsin, and thus the viral protease can be a selective target for antiviral agents.

A series of protease inhibitors has been recently licensed to treat HIV infections in AIDS patients. The protease inhibitors act directly on the target enzyme and do not require metabolic activation. Clinical studies suggest that the protease inhibitors are generally superior to the established nucleoside analogs for HIV treatment. Ritonavir is more than 500 times more selective for the HIV viral protease than for other cellular aspartic proteases, and suppresses HIV replication effectively, increasing CD4⁺ cell counts. The oral bioavailability of ritonavir is up to 70%, the highest

among the approved retrovirus specific protease inhibitors. Saquinavir is another type of HIV protease inhibitor. It has poor bioavailability, only 4%, but has shown durable antiviral activity in vivo with 50,000-fold lower affinity for cellular protease. Reported toxicities of the protease inhibitors include loose stools or diarrhea and elevated transaminases. Protease inhibitors are metabolized in the liver, and this may be the cause of liver enzyme elevation. Protease inhibitors are exclusively used to treat HIV infections, and have not yet been evaluated for veterinary practice.

Glycosylation Inhibitors

2-deoxy-D-glucose (2-dG) is a glucose analog interfering with the synthesis of oligosaccharides of viral surface glycoproteins. 2-dG inhibits a wide range of enveloped DNA and RNA viruses, especially orthomyxoviruses, paramyxoviruses, and herpesviruses. Viruses containing glycoproteins decorated with abnormal sugar moieties have decreased infectivity because of their inability to recognize specific cell receptors or to penetrate into cells. Clinical benefit has been claimed for topical application to initial lesions of gen-

ital herpes in women. This has not been confirmed in controlled trials.

Calves administered 20 mg/kg 2-dG daily IV had no protection against experimentally induced respiratory infectious bovine rhinotracheitis (IBR) infection, but ocular instillation markedly reduced the severity of experimental keratoconjunctivitis (Mohanty et al., 1980). A dosage of 10 mg/kg IV once a day appeared safe and protected calves against the mild clinical signs of bovine respiratory syncytial virus infection. The drug had no effect when administered after development of clinical signs or against a more severe respiratory tract illness such as bovine herpesvirus infection. 2-dG has potential application in the prophylaxis of canine distemper, equine influenza, and parainfluenza infections.

Sources and Potential Clinical Applications for Antiviral Drugs

Sources and potential application of antiviral drugs in veterinary medicine are shown in Tables 20.1–20.3.

Table 20.1. Commercially available antiviral drugs.

Generic name	Trade name	Manufacturer and formula
Acyclovir	Zovirax	Glaxo Wellcome, 5% ophthalmic ointment, 200 mg capsule, 400 mg or 800 mg tablet, 200mg/5 ml syrup in 1 pint, 500 mg/vial for IV
Amantadine	Symmetrel	Du Pont Pharma, 100 mg tablet, 50mg/5ml syrup
Amantadine	Symadine	Solvay, 100 mg tablet
2-deoxy-glucose 2-dG		ICN Pharmaceuticals, powder, 1 g, 5 g, 25 g
Famciclovir	Famvir	SmithKlein Beecham, 500 mg tablet
Fomivirsen	Vitravene	CIBA Vision, 0.25 ml ophthalmic solution
Foscarnet	Foscavir	Astra, 24 mg/ml in 250 ml or 500 ml for IV use
Ganciclovir	Cytovene	Hoffman-La Roche, 1000 mg capsule
Idoxuridine	Herplex	Allergan, 0.1% solution
Interferon- α 2a	Roferon-A	Roche, 3, 6, 36 $\times 10^6$ IU/vial, IV
Interferon- α N3	Alferon N	Purdue Frederick, 5 $\times 10^6$ U/vial, IV
Interferon- β	Betaseron	Berlex, 0.3 mg/vial, IV
Interferon- γ 1b	Actimmune	Genentech, 3 $\times 10^6$ U/0.5ml, IV
Lamivudine	Epivir	Glaxo Wellcome, 150 mg/tablet
Rimantadine	Flumadine	Forest Laboratories, 50 mg/5 ml syrup
Ribavirin	Virazole	ICN, 6 g/vial for reconstitution for aerosol
Ritonavir	Norvir	Abbott Laboratories, 100 mg capsules, 80 mg/ml 240 ml oral solution
Saquinavir	Invirase	Hoffman-La Roche, 200 mg capsule
Trifluridine	Viroptic	Burroughs Wellcome, 1% solution
Vidarabine	Vir-A	Parke-Davies, 3% ointment, FHV-1
Valaciclovir	Valtrex	Glaxo Wellcome, 500 mg capsule, zoster and genital herpes
Zalcitabine(ddC)	Hivid	Hoffman-La Roche, 0.375 mg tablet, 0.750 mg tablet
Zidovudine(AZT)	Retrovir	Glaxo Wellcome, 100 mg oral capsule, 50 mg/5ml syrup, 10 mg/ml solution for IV

Table 20.2. Topical antiviral drugs used in humans and their potential veterinary uses.

Drug	Human use	Potential Veterinary Use
Acyclovir	First genital herpes infections in otherwise healthy persons; limited mucocutaneous infections in immuno-compromised patients; Herpes zoster	Local herpes infections: bovine herpes mammillitis, equine coital exanthema, feline rhinotracheitis, viral keratoconjunctivitis
2-deoxy-D-glucose	Not approved	Local herpes infections (see above); bovine vulvovaginitis and keratoconjunctivitis
Fomivirsen	Cytomegalovirus retinitis (intravitreal)	Cytomegalovirus
Iodoxuridine	Herpes simplex keratitis	Same as deoxy-D-glucose; feline herpesvirus keratitis
Methisazone	Not used topically	Bovine vaccinia or pseudocowpox teat lesions
Foscarnet	Genital herpes	Same as deoxy-D-glucose
Trifluridine	Herpetic keratitis	Same as deoxy-D-glucose; drug of choice for feline herpesvirus keratitis
Ribavirin	Aerosol in animal model; for influenza and respiratory syncytial virus	Local herpes infections (not feline); vaccinia teat lesions
Vidarabine	Herpes keratitis	Local bovine herpes or vaccinia teat lesions

Future Prospects for Antiviral Chemotherapy

Even though viruses do not carry mechanisms *per se* for developing resistance to antiviral agents, viruses can undergo mutations to introduce changes on the viral enzymes or structural components. Resistant viruses are generally less virulent and poor in their transmission. However, in the face of immunosuppression, mutant viruses may proliferate favorably under the selective pressure of antiviral drugs. Rimantadine-resistant influenza A virus is as readily transmissible and as virulent in ferrets and humans as wild-type virus. Viruses resistant to nucleoside analogs may have developed mutations of their DNA polymerase and reverse transcriptase. Similarly, resistance to protease inhibitors may arise from mutations at the drug-binding sites. Resistance to one drug is usually accompanied by reduced susceptibility to drugs in the same class, though different classes of drugs may still retain antiviral effects to the resistant virus.

In this regard, combination drug therapy is an effective approach to some viral infections. Combination therapy has several advantages. First, it delivers the most efficient individual drugs simultaneously and may provide synergistic effects. Second, combination of two or more drugs may allow the dosage of individual drugs to be reduced, and thus dosage-dependent cytotoxicity may be minimized. Third, combination therapy may prevent emergence of drug resistance by using different antiviral drugs that have different mechanisms of action.

A study using AZT, ddI, and a protease inhibitor in

combination in AIDS clinical trials suggests that there is no interference in the kinetics among the individual drugs (Vanhove et al., 1997). AZT and IFN- α combination showed a favorable effect on CD4+ cell counts in symptomatic AIDS patients (Frissen et al., 1994). A synergistic effect was also observed for feline infectious peritonitis virus (FIPV) when 10 million units of IFN- α was combined with variable amounts of ribavirin. The combination produced an 80–200 fold increase in the effects of the individual drug alone (Weiss and Oostrom-Ram, 1989). When the same combination was tested against feline herpesvirus, the maximum inhibitory effect against virus replication was maintained despite an 8-fold reduction of the acyclovir dosage (Weiss, 1989).

Antiviral agents may have an antiproliferative influence on cells, which may lead to suppression of host immune responses. Heagy et al. (1991) found that AZT, ganciclovir, and ribavirin decrease mitogenesis, while acyclovir and ddI have no inhibitory effects on peripheral blood mononuclear cells in humans. Another study suggests that AZT, ddI, and ddC have no inhibitory effects on polymorphonuclear leukocytes (Roilides et al., 1990). Further studies are required to identify and characterize the immunosuppressive properties of the antiviral agents.

Protease inhibitors and antisense oligonucleotides are the “new generation” antiviral agents. These agents are highly selective and thus considerably reduce undesirable cytotoxicity. The development of highly specific antiviral drugs requires in-depth understanding

Table 20.3. Systemic antiviral agents in human clinical and experimental use, with suggested veterinary uses.

Drug	Human Use (Route)	Suggested Veterinary Use
Acyclovir	Systemic herpes prophylaxis, Varicella eye infections	Animal herpes viruses generally not very susceptible; equine herpes in foals
Amantadine	Influenza A prophylaxis, chronic hepatitis C (PO)	Equine influenza A prophylaxis
Foscarnet	Herpes simplex encephalitis (IM), Cytomegalovirus retinitis	Infectious bovine rhinotracheitis, feline rhinotracheitis, Aujeszky's disease prophylaxis, retroviral infections
Ganciclovir	Herpesvirus, especially cytomegalovirus retinitis	Cytomegalovirus
Interferon	Papillomavirus, chronic hepatitis B, chronic hepatitis C (IM, SC)	Feline herpes, feline leukemia (FeLV), feline infectious peritonitis, feline immunodeficiency virus (FIV)
Ribavirin	Influenza A and B, measles, hepatitis A, Lassa fever (IV), respiratory syncytial virus (PO, aerosol), hepatitis C (PO), hantavirus (IV)	Influenza, parainfluenza, bovine herpes virus, canine distemper, blue tongue, rotavirus, Marek's disease, feline infectious peritonitis, feline calicivirus
Saquinavir, zidovudine	HIV (PO)	Potentially for FIV, FeLV
Vidarabine	Disseminated herpes simplex, herpes zoster, herpes simplex encephalitis (IV)	Canine herpesvirus, etc., as for Foscarnet
Zidovudine (AZT)	Retrovirus, HIV (PO)	Retrovirus: FeLV, equine infectious anemia, FIV

of the replication cycles and molecular biology of viruses. The three-dimensional structure of viral proteins is elucidated by X-ray crystallographic studies, and drugs may be designed by computer simulation to delineate interactions of the protein with other molecules. Chemical compounds are then synthesized based on this information. Design of antisense oligonucleotides as antiviral drugs also requires two dimensional analysis of targeted mRNA structure. Poliomyelitis virus, influenza virus, and hepatitis C virus are among the targeted viruses for such sophisticated approaches.

Antiviral chemotherapy is still in its infancy compared to the development of other antimicrobial chemotherapies. Nonetheless, progress is being made rapidly and will eventually lead to the discovery of safe, specific, and effective antiviral drugs.

Bibliography

- Araujo FG, Remington JS. 1991. Synergistic activity of azithromycin and gamma interferon in murine toxoplasmosis. *Antimicrob Agents and Chemother* 35:1672.
- Babiuk LA, et al. 1987. Use of recombinant bovine IFN- α 1 in reducing respiratory disease induced by bovine herpesvirus type 1. *Antimicrob Agents and Chemother* 31:752.
- Ben-Nathan D, et al. 2003. Prophylactic and therapeutic efficacy of human intravenous immunoglobulin in treating West Nile virus infection in mice. *J Infect Dis* 188:5.
- Collinson PN, et al. 1994. Isolation of equine herpesvirus type 2 (equine gammaherpesvirus 2) from foals with keratoconjunctivitis. *J Am Vet Med Assoc* 205:329.
- Dennis MJ, et al. 1991. Effects of recombinant human alpha A interferon in gnotobiotic calves challenged with respiratory syncytial virus. *Res Vet Science* 50:222.
- De Mari K, et al. 2004. Therapeutic effects of recombinant feline interferon-omega on feline leukemia virus (FeLV)-infected and FeLV/feline immunodeficiency virus (FIV)-coinfected symptomatic cats. *J Vet Intern Med* 18:477.
- Frissen PH, et al. 1994. Zidovudine and interferon- α combination therapy versus zidovudine monotherapy in subjects with symptomatic human immunodeficiency virus type 1 infection. *J Infect Dis* 169:1351.
- Fox JL. 1998. FDA approves first antisense drug for CMV retinitis. *ASM News* 64:679.
- Fox LK, et al. 1990. The effect of interferon-gamma intramammary administration on mammary phagocyte function. *J Vet Med B* 37:28.
- Greene CE. 1998. Ocular infections. In: Greene CE, Ed. *Infectious Diseases of the Dog and Cat*, 2nd ed. Philadelphia: WB Saunders. p658.
- Heagy W, et al. 1991. Inhibition of immune functions by antiviral drugs. *J Clin Invest* 87:1916.
- Julander JG, et al. 2005. Treatment of West Nile virus-infected mice with reactive immunoglobulin reduces fetal titers and increases dam survival. *Antiviral Res* 65:79.
- Lee JB, et al. 1995. Immunological response to recombinant VP8* subunit protein of bovine rotavirus in pregnant cattle. *J Gen Virol* 76:2477.
- McCaw DL, et al. 2001. Immunomodulation therapy for feline leukemia virus infection. *J Am Anim Hosp Assoc* 37:356.
- Morrey JD, et al. 2004. Effect of interferon-alpha and interferon-inducers on West Nile virus in mouse and hamster animal models. *Antivir Chem Chemother* 15:101.

- Murray MJ, et al. 1998. Neonatal equine herpesvirus type 1 infection on a Thoroughbred breeding farm. *J Vet Intern Med* 12:36.
- Nasissie MP, et al. 1997. Effects of valciclovir in cats infected with feline herpesvirus 1. *Am J Vet Res* 58:1141.
- Pennington JE. 1990. Newer uses of intravenous immunoglobulin as anti-infective agents. *Antimicrob Agents and Chemother* 34:1463.
- Povey RC. 1978. Effect of orally administered ribavirin on experimental feline calicivirus infection in cats. *Am J Vet Res* 39:1337.
- Rees WA, et al. 1997. Amantadine and equine influenza: pharmacology, pharmacokinetics and neurological effects in the horse. *Equine Vet J* 29:104.
- Rees WA et al. 1999. Pharmacokinetics and therapeutic efficacy of rimantadine in horses experimentally infected with influenza virus A2. *Am J Vet Res* 1999 60:888.
- Roilides E, et al. 1990. Effects of antiretroviral dideoxynucleosides on polymorphonuclear leukocyte function. *Antimicrob Agents and Chemother* 34:1672.
- Rosenquist BD, Allen GA. 1990. Effect of bovine fibroblast interferon on rhinovirus infection in calves. *Am J Vet Res* 51:870.
- Rueckert RR. 1996. Picornaviridae: the viruses and their replication. In: Fields BN, et al., eds. *Fields Virology*, 3rd Ed. New York: Lippincott-Raven, p609.
- Samorek-Salamonowicz E, et al. 1987. Effect of acyclovir on the replication of turkey herpesvirus and Marek's disease virus. *Res Vet Sci* 42:334.
- Schwens A, et al. 1989. Protection of cattle from infection with vaccinia virus by bovine interferon alpha C. *Vet Record* 125:15.
- Smyth NR, et al. 1994a. Effect of 3'-azido-2', 3'-deoxythymidine in domestic cats. *Res Vet Sci* 57:220.
- Smyth NR, et al. 1994b. Susceptibility in cell culture of feline immunodeficiency virus to eighteen antiviral agents. *J Antimicrob Chemother* 34:589.
- Sordillo LM, Babiuk LA. 1991. Controlling acute *Escherichia coli* mastitis during the periparturient period with recombinant bovine interferon gamma. *Vet Microbiol* 28:189.
- Swenson CL, et al. 1990. Age related differences in pharmacokinetics of phosphonoformate in cats. *Antimicrob Agents and Chemother* 34:871.
- Tavares L, et al. 1987. 3'-azido-3'-deoxythymidine in feline leukemia virus-infected cats: a model for therapy and prophylaxis of AIDS. *Cancer Res* 47:3190.
- Uckun FM, et al. 2003. In vivo antiretroviral activity of stavudine in chronically feline immunodeficiency virus-infected cats. *Antimicrob Agents Chemother* 47:1233.
- Vanhove GF, et al. 1997. Pharmacokinetics of saquinavir, zidovudine, and zalcitabine in combination therapy. *Antimicrob Agents and Chemother* 41:2428.
- Vilček J, Sen GC. 1996. Interferons and other cytokines. In: Fields BN, et al., eds. *Fields Virology*, 3rd Ed. New York: Lippincott-Raven, 375.
- Weiss RC. 1989. Synergistic antiviral activities of acyclovir and recombinant human leukocyte (alpha) interferon on feline herpesvirus replication. *Am J Vet Res* 50:1672.
- Weiss RC, Oostrom-Ram T. 1989. Inhibitory effects of ribavirin alone or combined with human alpha interferon on feline infectious peritonitis virus replication in vitro. *Vet Microbiol* 20(3):255-265.
- Weiss RC, et al. 1990. Effect of interferon or *Propionibacterium acnes* on the course of experimentally induced feline infectious peritonitis in specific-pathogen-free and random-source cats. *Am J Vet Res* 51:726.
- Wilkins PA, et al. 2005. Pharmacokinetics of acyclovir in adult horses. *J Vet Emerg Crit Care* 15:174-178.
- Zeidner NS, et al. 1990. Alpha interferon (2b) in combination with zidovudine for the treatment of presymptomatic feline leukemia virus-induced immunodeficiency syndrome. *Antimicrob Agents and Chemother* 34:1749.

Section III

Special Considerations

Prophylactic Use of Antimicrobial Agents and Antimicrobial Chemotherapy for the Neutropenic Patient

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Infectious diseases of bacterial etiology occur because the host has been exposed to a sufficient number of organisms that have the capability of causing disease (e.g., salmonellosis), or because there has been an assault on the host's specific and non-specific defense mechanisms (e.g., traumatic injury, surgical procedure, dramatic change in environment, or neutropenia). These assaults on physical barriers or defense mechanisms may render the host susceptible to infection from its normal flora or from other organisms with which it might come in contact. It is not uncommon for a clinician, recognizing the assault on the host's defenses, to initiate antimicrobial chemotherapy in an effort to ward off the pending infection or to assist the host in combating the infection until its natural defenses have recovered. When such therapy is initiated in an animal that is about to undergo a surgical procedure or has experienced a traumatic injury and the clinician wants to protect against infection, such therapy is referred to as prophylaxis. When an antimicrobial agent is administered to a herd or flock of animals that are at risk of a disease outbreak due to transport, crowding or some other exposure to infectious agents, the therapy is referred to as metaphylaxis. When therapy is initiated in a neutropenic animal, with or without an ongoing infection, the use of antimicrobial agents may be considerably different from that in animals with intact defense mechanisms. This chapter discusses the prophylactic use of antimicrobial agents in a herd situation, prior to a surgical procedure, and in neutropenic animals.

Prophylactic or Metaphylactic Use of Antibiotics in Livestock

Robert D. Walker and Steeve Giguère

The prophylactic or metaphylactic use of antimicrobial agents has had a tremendous impact on the prevention and control of infectious diseases in veterinary medicine particularly in farm animals. However, it has not been without its drawbacks, the most obvious being the risk of selection for resistant organisms. To minimize the risk of selecting for resistant organisms there are a few guidelines that should be followed when using antimicrobial agents prophylactically. These include:

1. Knowledge of the pathogen(s) putting the patient at risk.
2. Knowledge of the antimicrobial agents to which the suspected pathogen(s) are susceptible.
3. Initiation of therapy before the onset of infection to ensure there are adequate drug concentrations at the site of concern before the bacterial pathogen reaches sufficient concentration to cause disease. For herds or flocks, this should be at the time of exposure or at the first signs of a disease outbreak before it has fully manifested itself.
4. The duration of prophylaxis should be as short as possible, consistent with efficacy, and should be used only where its efficacy is clearly established.
5. The dosage must be the same as that used therapeutically.

Antimicrobial agents are often administered prophylactically when young animals (pigs, calves) are moved from breeding to growing areas, because disturbances in microbial flora and physiology and the

sudden exposure to pathogens can spark outbreaks of infectious disease. Because of the disadvantages, the use of antimicrobial drugs for such purposes should be replaced, wherever possible, by adequate preventive husbandry practices. Addressing the immune status of the animals, the stress to which the animals are exposed and the pathogen load in the animal's environment may all contribute to decreasing the incidence of infection. For example, Berge et al. (2005) investigated the influence of prophylactic antibiotics on health and performance in 120 preweaned dairy calves. The most important factor associated with morbidity and mortality was inadequate transfer of passive immunity through colostrum. In-feed antibiotics delayed the onset of morbidity, decreased overall morbidity, and increased weight gain. However, rearing the calves that did not receive adequate transfer of passive immunity was more difficult and labor-intensive than raising calves with adequate immunoglobulin concentrations, despite the use of prophylactic antibiotics. Many antimicrobial agents used as growth promoters also have an impact on infectious disease prevention. The use of antimicrobial agents as growth promoters and their effects on disease prophylaxis is discussed in Chapter 24.

Metaphylaxis is employed extensively in veterinary medicine where herd health is at risk. Examples of metaphylaxis include preemptive medication in a dairy herd in the form of dry-cow therapy (Chapter 31). Such drug use is based on knowledge that disease is present in the population and will continue to affect susceptible individuals. Preemptive medication of the herd or individual reduces shedding of pathogens. The concept of herd medication is to treat the whole group at risk rather than individuals. Typical examples are (1) giving drugs at prophylactic concentrations to prevent swine dysentery (Chapter 34), (2) using "blitz" therapy with intramammary penicillin G to eradicate *Streptococcus agalactiae* infection from a cow herd, (3) ensuring specified disease-free pigs by the medicated-early-weaning system, and (4) mass medication on arrival at the feedlot to decrease the incidence of bovine respiratory disease (Chapter 30). A meta-analysis of 107 field trials in cattle indicated that mass medication with oxytetracycline or tilmicosin on arrival at the feedlot consistently reduced morbidity, but effects on mortality and performance were inconsistent (Van Donkersgoed, 1992). In one study, medication with tulathromycin was more effective in preventing natural outbreaks of bovine respiratory disease than tilmi-

cosin (Godinho et al., 2005). However, the selection of an antimicrobial agent for prophylaxis or metaphylaxis depends not only on efficacy, but also on overall cost/benefit analysis. For example, one study comparing the prophylactic efficacy of tilmicosin and oxytetracycline found that there was a net economic advantage in using oxytetracycline because of lower cost, even though tilmicosin was significantly more effective in preventing undifferentiated fever (Schunich et al., 2002).

The use of prophylactic antibiotics in veterinary medicine has also been shown to have adverse effects on some animals. For example, the routine use of neomycin intrauterine infusions to prevent postparturition metritis in cows has been shown to have an adverse effect on subsequent fertility. Further, concurrent intrauterine infusions of gentamicin in inseminated mares adversely affects their ability to conceive. Tetracyclines administered via drinking water to feedlot calves have been associated with increased mortality (Martin et al., 1982). Examples of well-established prophylactic use of antimicrobial drugs are shown in Table 21.1.

Bibliography

- Berge ACB, et al. 2005. A clinical trial evaluating prophylactic and therapeutic antibiotic use on health and performance of preweaned calves. *J Dairy Sci* 88:2166.
- Godinho KS, et al. 2005. Efficacy of tulathromycin in the treatment and prevention of natural outbreaks of bovine respiratory disease in European cattle. *Vet Ther* 6:122.
- Martin SW, et al. 1982. Factors associated with mortality and treatment cost in feedlot calves: the Bruce County beef project, years 1978, 1979, 1980. *Can J Comp Med* 46:341.
- Schunich OC, et al. 2002. A comparison of prophylactic efficacy of tilmicosin and a new formulation of oxytetracycline in feedlot calves. *Can Vet J* 43:355.
- Van Donkersgoed J, et al. 1992. Meta-analysis of field trials of antimicrobial mass medication for prophylaxis of bovine respiratory disease in feedlot cattle. *Can Vet J* 33:786.

Antimicrobial Prophylaxis for Surgery

Steeve Giguère and Robert D. Walker

The implementation of prophylactic antimicrobial use to reduce the incidence of surgical site infection was a major milestone in the history of surgery. However, antimicrobial use does not replace aseptic technique

Table 21.1. Selected examples of antimicrobial prophylaxis or metaphylaxis in large animals.

Species	Disease/Purpose	Drugs	Duration	Comments
Cattle	Pneumonia of feedlot cattle	Oxytetracycline, florfenicol, tilmicosin, tulathromycin	1-3 doses	Treat upon arrival at feedlot.
	Dry-cow therapy	Many	Single dose	Intramammary infusion.
Swine	Leptospirosis	Oxytetracycline, tilmicosin	Single dose	Eradicates urinary shedding.
	Swine erysipelas	Penicillin, long-acting	Single dose	Treat pigs at risk.
	Atrophic rhinitis in pigs	Oxytetracycline	First weeks of life	
	Swine dysentery	Tiamulin, valnemulin, lincomycin	Varies with drug	
	Proliferative enteropathy	Tylosin, lincomycin, tiamulin, valnemulin	Varies with drug	
Horses	Clostridial enteritis	Salinomycin*	Prolonged	Administered in feed.
	Strangles	Penicillin	Depends on duration of exposure	Treat horses at risk before development of lymphadenopathy.

*No longer approved for use in the US as of January 2006.

and adherence to proper surgical principles such as minimizing trauma and hemorrhage, using adequate instrumentation, careful choice of suture material and implants, debriding devitalized tissues, and minimizing dead space. Although the benefit of proper prophylactic antimicrobial use prior to surgery is indisputable, unrestricted prophylactic use of antimicrobial agents may result in an increase risk of superinfection, development of resistant microorganisms, increased cost of hospitalization, and increased incidence of side effects for the host. Therefore, strict adherence to simple principles must be followed for optimal perioperative antimicrobial use.

The principles upon which drugs are used prophylactically to prevent surgical infections in animals are for the most part based on human studies because of the paucity of randomized veterinary trials. The selection and duration of antimicrobial prophylaxis should have the smallest impact possible on the normal bacterial flora of the patient and the microbiologic ecology of the hospital. This section summarizes the current state of knowledge on prophylactic use of antimicrobial agents for the prevention of surgical site infections in animals.

Risk Factors for the Development of Surgical Site Infections

All surgical wounds are contaminated at some point. Fortunately, infection at the site of surgery is the exception rather than the rule. Incisional site infections usually develop within 30 days of the procedure, or within one year if an implant was left in place. The de-

velopment of infection results from interactions between the nature and extent of microbial contamination, the virulence of microorganisms, the integrity of host innate and adaptive defense mechanisms, and factors that relate to the surgery itself.

A few studies have attempted to identify risk factors that influence infection rate in veterinary medicine. Epidemiologic evaluation of postoperative infections in 239 dogs and cats showed that intact males and animals with concurrent endocrinopathy are at higher risk of postoperative wound infection (Nicholson et al., 2002). Total surgery and anesthesia time are also well established risk factors in dogs and cats (Brown et al., 1997; Beal et al., 2000; Nicholson et al., 2002). One epidemiologic study of 1255 dogs and cats found that the risk of infection for animals undergoing a 90-minute procedure is twice as high as that of animals undergoing a 60-minute procedure, and the risk doubles for each additional hour of surgery thereafter (Brown et al., 1997). Similarly, equine orthopedic surgeries longer than 90 minutes are 3.6 times more likely to develop a surgical site infection than shorter procedures (MacDonald et al., 1994). In addition, the number of complications at the site of ventral celiotomy in horses is twice as high in surgeries lasting longer than two hours than in those of shorter duration (Wilson et al., 1995). Preparation of the surgical site is also important. For example, surgical sites clipped before anesthetic induction in dogs and cats are three times more likely to become infected than sites clipped after induction (Brown et al., 1997).

Additional risk factors for surgical site infections

Table 21.2. Classification of operative wounds based on the likeliness of bacterial contamination and associated risk of surgical site infection.

Classification	Criteria	Approximate Risk (%)
Clean	Elective. Nontraumatic. Primarily closed. No inflammation encountered. No break in aseptic technique.	<5
Clean-contaminated	Respiratory, alimentary, biliary, and genitourinary tracts not entered. Urgent or emergency case that is otherwise clean. Elective opening of respiratory, gastrointestinal, biliary, or genitourinary tract with minimal contamination and no encounter with infected urine or bile.	5-10
Contaminated	Minor break in technique. Nonpurulent inflammation. Gross spillage from gastrointestinal tract. Entry into biliary or genitourinary tract in the presence of infected bile or urine. Major break in technique.	10-20
Dirty	Penetrating trauma <4 hours old. Chronic open wounds to be grafted or covered. Purulent inflammation encountered during the procedure (e.g., abscess). Preoperative perforation of respiratory, gastrointestinal, biliary or genitourinary tract. Penetrating trauma >4 hours old.	>20

Adapted from Cruise and Ford, 1980.

recognized in humans include, among others, advancing age, obesity, corticosteroid therapy, chronic inflammation, the use of electrocautery, the use of braided/multifilament suture material, and severe concurrent illnesses. Some of these risk factors may also be valid in veterinary medicine. For example, the incidence of incisional complications for horses undergoing emergency surgery for acute abdominal disease (39%) is significantly higher than that of horses undergoing elective abdominal surgeries (7%) (Wilson et al., 1995).

Patient Selection

Recommendations for antimicrobial prophylaxis for surgery in veterinary medicine are based on the extent of operative contamination as predicted by the National Research Council wound classification system (Table 21.2). This classification, developed in people, may not be totally accurate in veterinary surgery, and its accuracy may vary according to the type of procedure. For example, in equine abdominal surgery, performing an enterotomy or intestinal resection does not influence the incidence of surgical site infection (Kobluk et al., 1989; Phillips and Walmsley, 1993). In contrast, there is a strong association between wound classification and the risk of surgical site infec-

tion for equine orthopedic procedures, where a clean-contaminated procedure is approximately 24 times more likely to develop a postoperative infection than a clean procedure (MacDonald et al., 1994).

Antimicrobial drugs are highly effective and necessary in preventing certain postoperative infections and should be used in surgical procedures where infection rates associated with a particular procedure exceed 5%. These typically include patients undergoing clean-contaminated or contaminated procedures. Prophylactic antimicrobials are not warranted for most clean surgical procedures because the risk of contamination is low. However, prophylactic antimicrobials are recommended for those clean procedures in which an implant is placed, or when an infection would be catastrophic to the outcome (e.g., total hip replacement) (Dunning, 2003). Prophylactic antimicrobials may also be indicated for clean surgical procedures in patients with concurrent debilitating diseases and in animals receiving immunosuppressive doses of corticosteroids.

Although these principles were originally borrowed from studies in people, there are now several studies in dogs, cats, horses, and cattle indicating that prophylactic antimicrobials provide no benefit for clean surgical

procedures (Holmberg, 1985; Vasseur et al., 1985; Klein and Firth, 1988a; MacDonald et al., 1994; Brown et al., 1997). On the other hand, studies in animals have demonstrated the benefit of prophylactic antimicrobials in clean-contaminated or contaminated procedures (Haven et al., 1992; Brown et al., 1997). By definition, dirty surgical procedures require therapeutic rather than prophylactic administration of antimicrobial agents and the guidelines of antimicrobial prophylaxis for surgery do not apply.

Antimicrobial Drug Choice

The selection of a prophylactic antibacterial drug must be based on the microorganisms most likely to contaminate the surgical site, the known activity of the drug against those microorganisms, low incidence of adverse effects, cost, pharmacokinetics of the drug in the species of interest, and pharmacodynamic indices associated with a favorable clinical and microbiological outcome. The use of newer broad-spectrum drugs should be avoided in surgical prophylaxis to decrease emergence of bacterial isolates that are resistant to these vanguard therapeutic agents (Bratzler et al., 2005).

In dogs and cats, as in people, cefazolin is the prophylactic antimicrobial of choice for most procedures, owing to its activity against most surgical wound pathogens, affordable cost, and minimal adverse effects (Dunning, 2003; Nichols et al., 2005). The microorganisms most commonly associated with orthopedic and abdominal surgical site infections in horses are Enterobacteriaceae (Moore et al., 1992). Therefore, it is common practice to administer gentamicin, in addition to either penicillin or cefazolin, to broaden the Gram-negative spectrum when antimicrobial prophylaxis is indicated in equine patients. In ruminants, penicillin or ceftiofur are commonly used for perioperative antimicrobial prophylaxis. Both drugs have distinct advantages and disadvantages. Penicillin offers the advantage of being more active than ceftiofur against *Arcanobacterium pyogenes* and many anaerobic pathogens commonly isolated from ruminants. Unfortunately, the duration of withdrawal time for milk and meat is a major disadvantage, and penicillin is not active against most Gram-negative bacterial isolates, e.g., Enterobacteriaceae. Conversely, ceftiofur has good activity against most Gram-negative pathogens isolated from ruminants. When used as labeled, ceftiofur sodium has no withdrawal time and ceftiofur hy-

drochloride has only a two-day withdrawal time for meat and no withdrawal time for milk.

Although the antimicrobial agents mentioned above often represent the default choice for prophylaxis in each species, clinicians must continue to evaluate current literature and carefully examine in vitro susceptibility patterns of bacterial isolates within their own institution or animal population. Emergence of resistance in bacterial pathogens associated with nosocomial surgical site infections has been reported in both large and small animal veterinary hospitals.

Timing and Duration of Antimicrobial Prophylaxis

The goal of antimicrobial prophylaxis is to achieve serum and tissue drug concentrations greater than MIC for microorganisms likely to be encountered for the entire duration of the surgery. Prophylactic antimicrobials should be administered at least 30 minutes but no more than 60 minutes before a surgical incision, so that they are adequately concentrated in tissues at the time of potential contamination. As early as 1961, it was demonstrated that incisions contaminated with *Staphylococcus aureus* could not be distinguished from uncontaminated controls when antimicrobial agents were administered before the incision (Burke, 1961). In the same study, antimicrobial agents were effective in minimizing severity of infection when administered no later than three hours after bacterial contamination. Since then, multiple studies in human medicine have shown that administration of the first antimicrobial dose after surgery results in surgical site infection rates almost identical to those of patients who did not receive prophylactic antimicrobials (Stone et al., 1976; McDonald et al., 1998). Administration of antimicrobial agents should be repeated intra-operatively if the surgical procedure lasts two half-lives after the first dose, to ensure adequate drug concentrations until wound closure (Bratzler et al., 2005). The half-life of cefazolin is slightly less than one hour in dogs and horses. The half-life of IV potassium or sodium penicillin and gentamicin in horses is approximately three hours, whereas procaine penicillin administered IM has a half-life of approximately 12 hours.

The optimal duration of antimicrobial prophylaxis in veterinary medicine is unknown. The vast majority of published evidence in human medicine demonstrates that antimicrobial prophylaxis after wound closure is unnecessary (Aber and Thore, 1991; Meijer et

al., 1990). Prolonged use of prophylactic antimicrobial agents is associated with emergence of resistant bacteria and is more likely to result in adverse effects (Harbarth et al., 2000). Based on published data, current recommendation from the National Surgical Infection Prevention Project is that prophylactic antimicrobial agents should be discontinued within 24 hours of the end of surgery (Bratzler et al., 2005). These guidelines should be followed in veterinary medicine as well. Consistent with findings in people, a single preoperative dose of penicillin prior to rumenotomy in cattle is as effective in preventing post-surgical complications as a seven-day course of the same antibiotic (Haven et al., 1992). It must be emphasized, however, that principles of perioperative surgical prophylaxis do not apply to dirty surgical procedures. Antimicrobial administration in these procedures is therapeutic rather than prophylactic and a longer course of therapy may be indicated. For example, the surgical infection rate in calves with complicated umbilical hernia is significantly lower after a four-day course of antimicrobials compared to calves treated for only one day (Klein and Firth, 1988b).

Bibliography

- Aber C, Thore M. 1991. Single versus triple dose antimicrobial prophylaxis in elective abdominal surgery and the impact on bacterial ecology. *J Hosp Infect* 18:149.
- Beal MW, et al. 2000. The effects of perioperative hypothermia and the duration of anesthesia on postoperative wound infection rate in clean wounds: a retrospective study. *Vet Surg* 29:123.
- Bratzler DW, et al. 2005. Antimicrobial prophylaxis for surgery: an advisory statement from the National Surgical Infection Prevention Project. *Am J Surg* 189:395.
- Brown DC, et al. 1997. Epidemiologic evaluation of postoperative wound infections in dogs and cats. *J Am Vet Med Assoc* 210:1302.
- Burke JF. 1961. The effective period of preventive antibiotic action in experimental incisions and dermal lesions. *Surgery* 50:161.
- Cruise PG and Ford R. 1980. The epidemiology of wound infection. A 10-year prospective study of 62,939 wounds. *Surg Clin North Am* 60:27.
- Dunning D. 2003. Surgical wound infection and the use of antimicrobials. In Slatter DH (ed). *Textbook of Small Animal Surgery*, 3rd edition. Philadelphia: Elsevier. p113.
- Harbarth S, et al. 2000. Prolonged antibiotic prophylaxis after cardiovascular surgery and its effect on surgical site infections and antimicrobial resistance. *Circulation*. 101:2916.
- Haven ML. 1992. Effects of antibiotic prophylaxis on postoperative complications after rumenotomy in cattle. *J Am Vet Med Assoc* 200:1332.
- Holmberg DL. 1985. Use of prophylactic penicillin in orthopedic surgery: a clinical trial. *Vet Surg* 14:160.
- Klein WR, Firth EC. 1988a. Infection rates in clean surgical procedures with and without prophylactic antibiotics. *Vet Rec* 123:542.
- Klein WR, Firth EC. 1988b. Infection rates in contaminated surgical procedures: A comparison of prophylactic treatment for one day or four days. *Vet Rec* 123:564.
- Kobluk CN et al. 1989. Factors affecting incisional complication rates associated with colic surgery in horses: 78 cases (1983-1985). *J Am Vet Med Assoc* 195:639.
- MacDonald DG, et al. 1994. An examination of the occurrence of surgical wound infection following equine orthopaedic surgery (1981-1990). *Equine Vet J* 26:323.
- McDonald M, et al. 1998. Single- versus multiple-dose antimicrobial prophylaxis for major surgery: a systematic review. *Aust N Z J Surg* 68:388.
- Meijer WS et al. 1990. Antibiotic prophylaxis in biliary tract surgery—current practice in The Netherlands. *Neth J Surg* 42:96.
- Moore RM, et al. 1992. Antimicrobial susceptibility of bacterial isolates from 233 horses with musculoskeletal infection during 1979-1989. *Equine Vet J* 24:450.
- Nichols RM, et al. 2005. Antibiotic prophylaxis in surgery—2005 and beyond. *Surg Infect* 6:349.
- Nicholson M, et al. 2002. Epidemiologic evaluation of postoperative wound infection in clean-contaminated wounds: A retrospective study of 239 dogs and cats. *Vet Surg* 6:577.
- Phillips TJ and Walmsley JP. 1993. Retrospective analysis of the results of 151 exploratory laparotomies in horses with gastrointestinal disease. *Equine Vet J* 25:427.
- Stone HH et al. 1976. Antibiotic prophylaxis in gastric, biliary and colonic surgery. *Ann Surg* 184:443.
- Vasseur PB, et al. 1985. Infection rates in clean surgical procedures: a comparison of ampicillin prophylaxis vs. a placebo. *J Am Vet Med Assoc* 187:825.
- Wilson DA, et al. 1995. Complications of celiotomy incisions in horses. *Vet Surg* 24:506.

Infections Associated with Neutropenia in the Dog and Cat

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Neutropenic animals are at increased risk of developing bacterial and fungal infections, and established infections in neutropenic patients are more difficult to treat. Such infections may be due either to organisms that are normally considered pathogenic or to opportunistic organisms that rarely cause disease in animals with normal defense mechanisms. This section on the management of infection in the neutropenic dog and

cat focuses on neutropenia resulting principally from impaired granulopoiesis and the attendant risk of opportunistic bacterial and fungal infection. Considerable attention has been given recently to the use of granulocyte colony-stimulating factor to increase neutrophil production, but antimicrobial therapy remains the cornerstone of managing neutropenia. A number of factors influence the risk and outcome of infection during neutropenia, but in most cases prompt therapy with appropriate antimicrobial agents will result in successful patient outcome. In cases of prolonged, severe neutropenia, patient management strategies must be extrapolated from the therapy of human neutropenic patients.

Causes of Neutropenia

Neutropenia may occur as a primary or secondary disorder, and as an isolated hematologic abnormality or as a feature of pancytopenia (Weiss, 1995; Brown and Rogers, 2001). Cyclic haematopoiesis of Grey Collies is a well-characterized inherited primary disorder that results in demonstrable neutropenia, but it is unlikely to be encountered in clinical practice. Belgian Tervuren dogs have a physiologic neutropenia, where neutrophil counts may be lower than the normal canine reference range, but there is no associated illness (Greenfield, 2000). Idiopathic neutropenia is occasionally seen in both dogs and cats. In some cases this is a result of immune-mediated mechanisms. Neutropenia may also occur secondary to infectious disease. Canine parvovirus-2 (CPV-2) and *Ehrlichia canis* are the principal infectious causes of neutropenia in the dog. Feline parvovirus (FPV), feline leukemia virus and feline immunodeficiency virus are the principal infectious causes of neutropenia in the cat. *Histoplasma capsulatum* may cause neutropenia in both dogs and cats secondary to bone marrow invasion.

Neutropenia may result from primary bone marrow neoplasia or from bone marrow involvement in metastatic disease. In either case there is likely to be concurrent anemia and thrombocytopenia. Sertoli cell tumors may cause pancytopenia due to paraneoplastic estrogen toxicosis.

Cytotoxic chemotherapy and radiation therapy for neoplastic and immune-mediated diseases predictably cause myelosuppression. The degree of resulting neutropenia varies with the agent and the dose administered. Other drugs with a known but unpredictable risk for causing neutropenia include estrogen and

phenylbutazone in dogs, and chloramphenicol, griseofulvin, propylthiouracil and methimazole in cats. Theoretically, any drug may be associated with an idiosyncratic reaction resulting in neutropenia. Such reactions have been reported with cephalosporins in dogs and cats, and with sulfonamides, captopril, phenobarbital, fenbendazole and albendazole in dogs. Autumn crocus intoxication may cause neutropenia; the toxic principle is colchicine.

Overwhelming bacterial infection may cause neutropenia in animals with normal granulopoiesis by exhausting marrow granulocyte reserves. Neutrophil consumption exacerbates neutropenia in animals with impaired granulopoiesis.

Infectious Complications of Neutropenia

Risk Factors

Factors determining the risk, severity, and outcome of infection during neutropenia include the severity and duration of neutropenia; disruption of natural barriers; defects in specific defenses; organisms involved; site of infection; presence and type of tumor and its biological stage; and age, performance status, and species of the host (Feld, 1989; Wade, 1994; Crawford et al., 2004; Sipsas et al., 2005).

The risk of infection is related to the degree of neutropenia, and neutropenia is graded to assist in predicting such risk (Veterinary Co-Operative Oncology Group, 2004). The risk of opportunistic infection occurs when the neutrophil count falls below $2.0 \times 10^9/L$. From 1.5 to $2.0 \times 10^9/L$ (grade 1 neutropenia), there is a marginal risk of infection. From 1.0 to $1.5 \times 10^9/L$ (grade 2 neutropenia), the risk is mild; and from 0.5 to $1.0 \times 10^9/L$ (grade 3 neutropenia), the risk is moderate. Animals with neutrophil counts $<0.5 \times 10^9/L$ (grade 4 neutropenia) have a high risk of infection; below $0.2 \times 10^9/L$ the risk of infection is very high. Below $0.2 \times 10^9/L$ there is still a relationship between worsening myelosuppression and adverse clinical consequences, but this is not reflected in the peripheral blood since any neutrophils released from the bone marrow immediately migrate into tissues. For all grades of neutropenia, a higher risk of infection is associated with a falling neutrophil count. These figures are based upon a classic study of humans with leukemia (Bodey et al., 1966). No such studies have been conducted in dogs or cats, but, based upon experimental studies with total body irradiation and clinical experience with veterinary cancer patients, these figures appear to be appli-

cable to the dog and cat (Couto, 1990; Abrams-Ogg et al., 1993; Veterinary Co-Operative Oncology Group, 2004).

The outcome of infection is related to the duration of neutropenia. Humans with neutropenia of short duration (<7 days) are unlikely to have severe infections that cannot be controlled with appropriate antimicrobial therapy. Infections accompanying neutropenia of moderate duration (7-14 days) are more difficult to manage. Infections in patients with prolonged neutropenia (>14 days) are even more difficult to manage, especially if the neutrophil count is $<0.2 \times 10^9/L$ (Feld, 1989). This, of course, is because antimicrobial agents act in concert with host defenses to eradicate infections.

The risk of infection during neutropenia is increased by disruption of natural physical barriers, and suppression of humoral and cell-mediated immunity. Natural barriers are disrupted, for example, with gastrointestinal damage during parvoviral infections and with anticancer chemotherapy, facilitating translocation of enteric bacteria. Intravenous catheterization and percutaneous biopsy procedures increase the risk of infection with skin organisms. Immunosuppression may accompany myelosuppression, because of the primary disease, anticancer therapy, and malnutrition. The risk of infection in neutropenic humans is greater if there is concurrent lymphopenia and monocytopenia.

The severity of infection is affected by the type of organism. Infections with Gram-positive organisms tend to be more easily managed than infections with Gram-negative organisms. The site of infection is also important in determining outcome. Bacteremia and pneumonia are more difficult to treat than soft tissue, gastrointestinal, or urinary tract infections.

In human cancer patients, the type of tumor and its stage are important factors. Infections are more likely to be severe in patients with acute compared to chronic hematologic malignancies, hematologic malignancies in relapse compared to those in remission, and hematologic malignancies compared to solid tumors. This is probably also true for dogs and cats. However, leukemias represent a larger proportion of hematologic malignancies in humans than in animals, and the comparative risk of infection for animals with lymphoma and mast cell tumors compared to solid tumors is not known. Older human patients and those with poor performance scores are at increased risk for infection and poor outcome. Host species is probably

important; for example, cats appear to be less susceptible than dogs to opportunistic infections during neutropenia. Finally, individuals vary in their innate susceptibility or resistance to infection (Neth et al., 2005).

Microbiology

Infections in neutropenic animals may occur with exogenous or endogenous organisms. Exogenous organisms are acquired from the environment. Nosocomial organisms are an important source of exogenous infections in neutropenic patients in human hospitals (Wade, 1994; Ellis, 2004), and probably represent a risk to neutropenic animals in veterinary hospitals (Warren et al., 2001).

Endogenous infections are caused by organisms from the host's own flora. The most important source is the intestinal tract. Other sources of endogenous infections include the oral cavity, skin, upper respiratory tract and lower urogenital tract. Exogenous and endogenous pathogens do not represent two entirely distinct groups of organisms, and the same organism may act as both an endogenous and exogenous pathogen for different individual animals.

Organisms causing infections in humans with neutropenia due to cytotoxic therapy have been extensively characterized (Sipsas et al., 2005). Gram-negative organisms, especially *E. coli*, *Klebsiella* spp., and *Pseudomonas aeruginosa*, were initially the most common causes. Gram-positive organisms, especially *Staphylococcus* spp., now account for up to 69% of infections. This change reflects the use of fluoroquinolones for antimicrobial prophylaxis and treatment and the increasing use of long-term central venous lines (Picazo, 2004).

Infections in dogs and cats with neutropenia secondary to cytotoxic therapy have not been as well-characterized. The majority of data have been anecdotally reported for myelosuppression in the dog. Similar to humans, the most frequent sites of infection appear to be the bloodstream (bacteremia) and the lung. Local cellulitis may occur, manifested as edema of one or more limbs. Other possible sites of infection include the oral cavity, gastrointestinal tract, genitourinary tract, heart and central nervous system.

Similar to the initial pattern of infection seen in humans, bacteremia is probably most often of intestinal origin and corresponds to the pattern of bacterial translocation seen in healthy dogs (Dahlinger et al.,

1997). Members of the Enterobacteriaceae, especially *E. coli* and *Klebsiella* spp., are most commonly isolated (Couto, 1990). *Pseudomonas* spp. are less frequently isolated, but have historically been associated with the most severe infections, because antibiotics effective against this organism were not initially available. Although the majority of bacteria in the intestinal tract are obligate anaerobes, they are not commonly the first invaders in opportunistic infection during neutropenia. *Clostridium difficile*-associated diarrhea may occur in neutropenic humans and dogs (Gor-schlüter et al., 2001; Weese and Armstrong, 2003). It is not known if neutropenia is a risk factor in addition to hospitalization, cytotoxic therapy and antimicrobial therapy; bacteremia is rare. Gram-positive bacteremia, usually with *Staphylococcus* spp. and *Streptococcus* spp., is less common than Gram-negative bacteremia, but more common than anaerobic bacteremia. Gram-positive bacteremia can arise from the skin, the intestinal tract or the oral cavity. Urinary tract infections are a possible source of bacteremia.

Pneumonia may occur as an opportunistic infection with upper respiratory flora or from translocation of intestinal bacteria. The same organisms are implicated as in bacteremia. Neutropenic dogs should probably also be considered at risk for *Bordetella bronchiseptica* pneumonia. Cats are likely at risk for pneumonia with *B. bronchiseptica* and *Pasteurella multocida*.

There is better documentation of bacterial infections secondary to parvoviral infections. Gram-negative organisms are believed to be the principal cause of sepsis; bacteremia and pneumonia may occur. *E. coli* was isolated from postmortem tissues of 88 of 98 dogs with CPV-2 infection (Turk et al., 1990). *E. coli* is also the most common isolate from post-mortem tissues of cats dying from FPV (Scott, 1987). In a report of bacterial colonization of IV catheters in 100 dogs with CPV-2 infection, 22 catheters became colonized with one or more organisms (Lobetti et al., 2002). *E. coli* and other enteric organisms were isolated from 13 catheters, there was one isolate each of *Staphylococcus* spp. and *Streptococcus* spp., and 18 isolates were of environmental origin. In another study of 43 dogs with CPV-2 infection, eleven dogs had asymptomatic bacteruria, ten of which had infections with *E. coli* and two with *Staphylococcus* spp. (Koutinas et al., 1998). In one study of experimental FPV infection, ten of thirty blood cultures were positive (Hammon and Enders, 1939). Isolates included *Pasteurella* spp., Gram-

negative bacilli, *Streptococcus* spp. and *Staphylococcus* spp. A *Bacillus* species was isolated in one culture along with a *Staphylococcus* sp. It is widely assumed, but not proven, that anaerobic bacteria contribute to bacteremia during parvoviral infections. It has been documented that *C. perfringens* proliferates in the intestinal tract of dogs with CPV-2 infection (Turk et al., 1992), but the role of the organism in sepsis is not known.

Local and systemic infections with *Aspergillus* spp., *Candida* spp., and less frequently organisms of the order *Mucorales* (zygomycosis) are important causes of disease in neutropenic humans (Wade, 1994; Brown, 2005). Risk factors for fungal infections are the same as those for bacterial infections. In addition, the risk of fungal infection increases with the duration of antibacterial therapy. Invasive fungal infections are not as common in neutropenic dogs and cats. This may be due in part to the use of less aggressive cytotoxic therapy for cancer. However, the risk for fungal infection is comparatively low even in experimental dogs with prolonged, severe neutropenia (Ehrensaft et al., 1979). Systemic candidiasis was reported in a pup with CPV-2 infection (Rodriguez et al., 1998). Pneumonia due to *Aspergillus* spp. has been reported in a dog following autologous bone marrow transplantation for treatment of lymphoma (Rosenthal, 1988) and in cats with FPV infection (Fox et al., 1978; Holzworth, 1987). Intestinal candidiasis associated with intensive antibiotic therapy occurred in three of six dogs with severe neutropenia induced by cytotoxic therapy (Abrams-Ogg et al., 1993). Intestinal candidiasis has also been reported as a complication of CPV-2 infection (Ochiai et al., 2000), and intestinal candidiasis, aspergillosis and zygomycosis have been reported as complications of FPV infection (Fox et al., 1978; Holzworth, 1987).

Patient Management

The majority of neutropenia that is managed in small animal medicine is of short duration (<7 days) or of mild to moderate severity. Animals with prolonged neutropenia usually have only mildly depressed counts. This reflects a tendency on the part of veterinarians to reduce or discontinue cytotoxic therapy when neutropenia develops, and to euthanize animals with severe pancytopenia that have a poor prognosis for prompt recovery. As veterinarians continue to employ more aggressive cytotoxic protocols and manage

dogs and cats with complex hematologic problems, severe and prolonged neutropenia may be more frequently encountered.

The risk of acquiring an exogenous infection is reduced by isolation. Neutropenic animals that do not require critical supportive care should be maintained at home. Cats should be kept indoors and dogs confined to the house and yard. In the hospital, contact with the general hospital population should be avoided. Hands should be thoroughly washed and laboratory coats changed before handling a neutropenic animal, and barrier nursing procedures, such as wearing gloves, gowns and isolation boots, should be considered for severe cases. The thermometer used for the neutropenic animal should not be used for other patients. A "low microbial diet" may be recommended for human patients with severe neutropenia (Wade, 1994; Wilson, 2002). The role of dietary pathogens has not been evaluated in neutropenic pet animals, but it is reasonable to recommend that only canned foods be offered and table scraps avoided in dogs and cats with severe neutropenia.

Antimicrobial therapy for neutropenic animals may be divided into three categories: (1) prophylactic therapy; (2) empirical treatment during febrile episodes; and (3) treatment of documented infection. Optimal protocols have not been completely defined for the management of infections in human neutropenic patients, and much less so for veterinary patients.

Prophylaxis

Prophylactic therapy is directed at the intestinal flora. The principal objective is "selective decontamination of the digestive tract" (Van der Waaij, 1988; Ellis, 2004). This refers to reduction of the aerobic Gram-negative organisms most often responsible for severe infections. The anaerobic population is left relatively undisturbed since it contributes to resistance to fungal overgrowth and colonization by exogenous organisms. A second objective of prophylactic therapy is to provide sufficient blood and tissue antimicrobial concentrations to contain an incipient bacterial infection.

Choices for prophylactic therapy are presented in Table 21.3. Neomycin and polymyxin B were first used but have been replaced by trimethoprim-sulfonamide combinations and fluoroquinolones in humans and dogs (Klastersky, 1989; Ellis, 2004). Amoxicillin and amoxicillin-clavulanate are not ideal choices because of activity against intestinal anaerobes. But they are

readily available, practical choices for cats, who often do not tolerate other choices and for whom prolonged use of fluoroquinolones is not recommended because of the risk of retinopathy. Cephalexin has also been used in dogs because of its activity against *E. coli* and *Klebsiella* spp., while causing less disturbance of the anaerobic population than amoxicillin. Amoxicillin and cephalexin also have good activity against susceptible Gram-positive organisms, which may be beneficial if surgical wounds are present.

Prophylactic therapy for human neutropenic patients has been reviewed (Wade, 1994; Donnelly, 1997; van de Wetering et al., 2005). Its use is controversial. The benefits are not clear, both with respect to reducing infection rates and in reducing mortality rates. In general, prophylactic therapy appears to be more beneficial in reducing infection rates in humans with neutropenia of greater severity and duration than in humans with mild to moderate neutropenia. In a study of veterinary cancer patients receiving vincristine-doxorubicin-cyclophosphamide chemotherapy, which resulted in neutropenic episodes of short duration with a median neutrophil count of $0.8 \times 10^9/L$, trimethoprim-sulfonamide prophylaxis reduced the incidence of antibiotic-responsive febrile episodes, presumably of infectious etiology, from 40% to 20% (Couto, 1990). The potential advantages of prophylactic therapy include reductions in infection rate, time to onset of infection, and rate of overwhelming sepsis. These benefits may facilitate home management of neutropenic animals and improve quality of life. Potential disadvantages include shifts in the host's flora, development of resistant organisms, adverse drug reactions, and expense (Wade, 1994; Williamson et al., 2002; Trepener, 2004; van de Wetering et al., 2005), although preventing sepsis is less expensive than treating it.

Antimicrobial prophylaxis in the asymptomatic patient should be considered whenever a neutrophil count of $\leq 0.5\text{--}1.0 \times 10^9/L$ is present or anticipated. We do not recommend routine prophylactic therapy during anticancer chemotherapy if the owner can closely observe the animal for signs of infection and if the anticipated neutropenia is of short duration, such as occurs with many commonly used protocols. Prophylactic therapy is specifically discouraged in cats because they have a better tolerance of neutropenia than dogs, but are more susceptible to antibiotic-induced gastrointestinal disorders (Kunkle et al.,

Table 21.3. Prophylactic oral antimicrobial therapy for the neutropenic dog and cat.

Antimicrobial	Dose	Comments
Diaminopyrimidine sulfonamides		
Trimethoprim-sulfamethoxazole (dogs)*	15 mg/kg (combined dose) q12h* 30 mg/kg (combined dose) q12-24h	Inexpensive. No prophylaxis against <i>Pseudomonas</i> spp. Risk for keratoconjunctivitis sicca and cutaneous, hematologic, and other immune-mediated abnormalities (Trepnier, 2004; Williamson et al., 2002). May retard marrow recovery following severe myelosuppression. As for trimethoprim-sulfamethoxazole, but more expensive.
Ormetoprim-sulfadimethoxine (dogs)	55 mg/kg on first day, then 27.5 mg/kg q24h	
Fluoroquinolones		
Enrofloxacin (dogs)*	5-10 mg/kg q12h* 10-20 mg/kg q24h*	Expensive. Lower dose effective for selective decontamination of the digestive tract. Doses >10 mg/kg needed to achieve tissue levels effective against <i>Pseudomonas</i> spp.
Ciprofloxacin (dogs)*	5-10 mg/kg q12h* 10-30 mg/kg q24h*	As for enrofloxacin.
Orbifloxacin (dogs)	2.5-7.5 mg/kg q24h	Expensive. Less well evaluated in neutropenia than enrofloxacin or ciprofloxacin.
Marbofloxacin (cats)*	2.75 mg/kg q24h*	As for orbifloxacin. Retinopathy has not been observed, but prolonged therapy with high doses of fluoroquinolones is not recommended in cats.
Difloxacin (dogs)	5 mg/kg q12h 5-10 mg/kg q24h	As for orbifloxacin.
β-Lactams		
Cephalexin (dogs)*	20 mg/kg q18h 30 mg/kg q12h*	Expensive. No prophylaxis against <i>Pseudomonas</i> spp.
Amoxicillin (cats)	10-20 mg/kg q12h	Inexpensive. No prophylaxis against <i>Pseudomonas</i> spp. Amoxicillin causes less intestinal disturbance than ampicillin.
Amoxicillin-clavulanate (cats)*	10-20 mg/kg q12h*	As for amoxicillin but more expensive. More activity than amoxicillin against <i>Staphylococcus</i> spp., <i>Klebsiella</i> spp., <i>Escherichia coli</i> , <i>Bacteroides</i> spp.
Combinations		
Fluoroquinolone + β -lactam	As above.	Reserved for animals with severe neutropenia.

Notes

*Drugs and dosages most commonly used by the authors.

Use of certain drugs for prophylaxis during neutropenia may be extra-label usage. Flexible labelling may specify once to twice daily use of certain drugs in dogs and cats depending upon the clinical situation. Once-daily use at the lower dose in the dose range probably results in selective decontamination of the digestive tract, although this has not been established with all drugs. Flexible dosing may specify twice-daily use when treating systemic infections, and may be more appropriate if goals include consistent tissue drug levels to treat incipient bacterial infections.

Doses adapted from Allen, 2004; Greene, 2005.

1995). Prophylaxis is, however, initiated in the asymptomatic animal when a neutrophil count $<0.5\text{--}1.0 \times 10^9/\text{L}$ is noted or anticipated during pre-treatment evaluation. Under these circumstances, chemotherapy is discontinued, and antimicrobial prophylaxis is continued until the animal is returned for its next chemotherapy treatment four to seven days later, at which point the neutrophil count has usually recovered. If the neutrophil count has not recovered sufficiently to resume chemotherapy, antimicrobial prophylaxis is discontinued if the neutrophil count is $>1.0\text{--}2.0 \times 10^9/\text{L}$.

If an animal has had a previous episode of chemotherapy-induced sepsis, then antimicrobial prophylaxis is given following the next treatment with the offending agent, but prophylaxis may be restricted to the period of five to 10 days post-treatment, i.e., the period when most post-chemotherapy neutropenia occurs.

Antimicrobial prophylaxis is also recommended if severe and prolonged neutropenia is anticipated, such as with pancytopenia caused by estrogen or phenylbutazone toxicosis. Prolonged neutropenia may also occur during the chronic phase of ehrlichiosis in dogs.

Ehrlichiosis is usually treated with tetracyclines. Doxycycline is less likely to disturb colonization than tetracycline and may be a superior choice in dogs with chronic neutropenia due to ehrlichiosis.

Antifungal prophylaxis, using topical decontamination with amphotericin B, nystatin and clotrimazole, has been practiced widely for many years in neutropenic humans. Despite these measures, the incidence of invasive fungal infections is increasing as anticancer therapy becomes more aggressive. This has led to the use of fluconazole and then itraconazole for systemic antifungal prophylaxis (Glasmacher et al., 1996; de Pauw, 2004). Routine antifungal prophylaxis is not recommended in veterinary medicine, but should be considered in experimental hematopoietic stem cell transplantation.

Empirical Treatment of Febrile Neutropenic Patients

Neutropenia itself does not cause clinical signs; these result from the underlying disease and infection. Most septic neutropenic animals will develop a fever because macrophages, rather than neutrophils, are largely responsible for the production of interleukin-1 and other endogenous pyrogens. Occasionally, inactivity, inappetance and tachycardia are the only signs of sepsis. This occurs mostly in older animals and in animals receiving corticosteroids, who may have blunted febrile responses. Septic animals may also present with vomiting, diarrhea, or septic shock. Local signs of inflammation are subtle or absent if granulopoiesis is impaired, and the site of infection may be difficult to determine. In many cases, it is impossible to document a suspected infection and fevers many remain unexplained (Hughes et al., 2002).

Body temperature should be monitored in the asymptomatic neutropenic animal and in the animal at risk for neutropenia. Depending upon perceived risk, this may vary from recording temperature when the animal shows signs of lethargy or inappetance to regular temperature recordings two to four times a day. Axillary temperature measurements facilitate home monitoring and minimize rectal trauma, and measure 0.5–1°C lower than rectal temperature measurements. The definition of pyrexia depends to some extent on baseline body temperatures obtained for an individual animal. In general, a rectal temperature >39°C in dogs and 39.2°C in cats should be regarded with suspicion and the animal either treated for sepsis or the temperature rechecked in several hours to de-

tect progressive elevation. A temperature above 39.5°C in most cases represents true fever.

A febrile episode or unexplained depression or inappetance in a neutropenic animal should be considered bacterial in origin until proven otherwise and antimicrobial therapy should be initiated promptly. The animal should be closely examined for any signs of inflammation, and an appropriate specimen collected for culture. If there is no obvious site of infection, blood cultures should be considered. Our protocol is to obtain two simultaneous samples for culture from different veins (Reller, 1994). Blood cultures are expensive, results take two to seven days to report, and they are often negative or do not alter initial therapy. For these reasons, blood cultures are not always performed during anticancer chemotherapy when the anticipated duration of neutropenia and fever is short, nor are they routinely performed in animals with parvoviral infections. Blood cultures are always recommended if the cause of neutropenia is not known or if the animal is very sick.

Additional tests may be performed in an effort to localize infection and determine the severity of illness. Recommended baseline measurements in hospitalized animals include serum glucose, urea, and electrolyte levels, and urine specific gravity. Thoracic radiographs may be considered as part of the minimum data base, and should always be obtained if the animal is coughing, dyspneic, or has a nasal discharge. Culture of airway (transtracheal or bronchoalveolar) lavage samples should be performed if there are radiographic signs of pneumonia. Normal thoracic radiographs, however, do not rule out pneumonia, and airway lavage cultures should be considered if the animal has signs of respiratory tract disease, is severely ill without localizing signs, or does not respond to antimicrobial therapy.

Urinalysis and urine culture are recommended if there are any signs of urinary tract infection, but therapy should not be delayed more than one to two hours (or less, depending upon the clinical status of the animal) while awaiting adequate urine production for collection. This recommendation applies to obtaining other cultures as well. Catheterization should be avoided because of the risk of introducing infection. If cystocentesis cannot be performed because of thrombocytopenia ($<20\text{--}50 \times 10^9/\text{L}$), a properly collected free catch sample submitted for quantitative culture will suffice. A serum chemistry profile, abdominal radiographs and/or abdominal ultrasound examination

are recommended if the animal is vomiting or has abdominal pain. All the preceding tests may be needed to characterize the illness if the cause of neutropenia is not known, if the animal is severely ill, or if there is no response to antimicrobial therapy.

Untreated infection may be rapidly fatal in neutropenic patients. Because of the likelihood that pyrexia is due to infection, and because neutropenic animals have died of sepsis with negative ante-mortem cultures, it is recommended to initiate empirical antimicrobial therapy while awaiting culture results, and, in most cases, to continue therapy in spite of negative results (Hughes et al., 2002; Rolston, 2004). Antimicrobial selection may be assisted by previous culture results (e.g., a dog with a history of recurrent urinary tract infection), localization and nature of the infection, clinical signs, Gram stain of body fluid (e.g., airway wash), and the antibiogram of a suspected pathogen. If there is a history of prophylactic therapy with a fluoroquinolone, a febrile episode is most likely due to a Gram-positive organism. Cultures of feces, the oral cavity, or the skin of an animal without clinical signs prior to the onset of neutropenia are not likely to yield useful information.

In many cases the choice of antimicrobial agents must be empirical. Numerous trials with various antibiotic combinations have been conducted in humans (Hughes et al., 2002; Picazo, 2004; Sipsas et al., 2005). Veterinary experience is much more limited. The antibiotics chosen should be bactericidal; parenteral; active against Enterobacteriaceae, *Pseudomonas* spp., and Gram-positive cocci; and minimally toxic to the bone marrow. Standard recommended drug doses should be employed. A representative selection of antibiotics is presented in Table 21.4. These protocols provide some activity against anaerobic organisms (except for imipenem-cilastatin and meropenem, which have broad-spectrum anti-anaerobe activity).

More complete therapy against anaerobic organisms is not recommended for initial therapy, since anaerobic infections are not common under conditions of neutropenia and such therapy may alter colonization of mucosal surfaces. Combination therapy has historically been preferred over therapy with a single agent in order to increase the antibacterial spectrum, take advantage of additive and synergistic effects while minimizing toxicity, and possibly to reduce the development of antimicrobial resistance. Most approaches have combined an aminoglycoside

antibiotic with a β -lactam antibiotic. Combination therapy with β -lactam antibiotics has been used as well in order to avoid aminoglycoside nephrotoxicity. This may also be accomplished by substituting a fluoroquinolone for an aminoglycoside. Although fluoroquinolones are considered broad-spectrum antimicrobial agents, in neutropenic patients they have limited activity against Gram-positive organisms. Fluoroquinolones are similar in spectrum to aminoglycosides, with excellent activity against Enterobacteriaceae and *Pseudomonas* spp. and limited activity against anaerobes. Single-agent therapy with ceftazidime, imipenem-cilastatin or meropenem is another option (Klastersk, 1997; Rolston, 2004). Cefoxitin has not been used as a single agent in humans presumably because of its lack of activity against *Pseudomonas* spp., but it has been used in animals, especially in cats and immature animals where fluoroquinolone therapy may not be appropriate.

For infections complicating the mild to moderate episodes of neutropenia usually encountered by veterinarians, the various protocols are probably of near equivalent efficacy. We generally use enrofloxacin plus cefazolin in canine cancer patients. Imipenem-cilastatin is our preferred choice for initial therapy in animals with sepsis associated with neutropenia of unknown cause.

Intravenous administration is preferred, to ensure rapid drug distribution, minimize tissue trauma and patient discomfort, and minimize bleeding in thrombocytopenic animals. IV catheterization is preferred to repetitive venipuncture, and is necessary for fluid therapy. However, there must be strict adherence to aseptic procedure during catheter placement. A sterile adhesive strip or plaster (e.g., Band-Aid®) should be placed over the skin entry site and the site bandaged. Injection ports should be cleansed with alcohol and allowed to dry before each injection. The catheter should be removed promptly and cultured if signs of phlebitis occur.

Drug toxicity should be considered during therapy. Animals receiving aminoglycosides should be monitored for evidence of nephrotoxicity (e.g., urinary casts, glucosuria, azotemia), especially when the duration of therapy is greater than five days. The order of aminoglycosides with respect to increasing nephrotoxicity (and decreasing cost) is netilmicin, amikacin, tobramycin and gentamicin. Fluoroquinolones should be avoided in animals less than six months old because

Table 21.4. Parenteral empirical antimicrobial therapy for the febrile neutropenic dog or cat.

Drug(s)	Comments
Combinations	
Aminoglycoside + cefazolin or cephalothin (1st-generation cephalosporins)	Once commonly used in veterinary medicine for cancer patients. Once commonly used in human medicine. Relatively inexpensive. Spectrum may not cover <i>Pseudomonas</i> spp. Cephalosporin may increase risk of nephrotoxicity.
Aminoglycoside + ampicillin	Commonly used in veterinary medicine for patients with parvoviral infections. Relatively inexpensive. Spectrum may not cover <i>Pseudomonas</i> or <i>Staphylococcus</i> . Increased activity against anaerobes over aminoglycoside + 1st generation cephalosporin. More likely to disturb colonisation resistance. Can inhibit β -lactamase activity by using ampicillin-sulbactam (parenteral substitute for amoxicillin-clavulanate).
Aminoglycoside + anti-pseudomonal penicillin or ceftazidime (3rd-generation cephalosporin)	Commonly used in human medicine for cancer patients. More expensive than above combinations. Synergy against <i>Pseudomonas</i> and <i>Enterobacteriaceae</i> . Less activity against Gram-positive organisms. Can inhibit β -lactamase activity by using ticarcillin-clavulanate or piperacillin-tazobactam.
Fluoroquinolone substituted for aminoglycoside in above combinations	Less well evaluated than aminoglycoside combinations. More expensive than aminoglycoside. Combinations more likely to be additive than synergistic. Avoids aminoglycoside nephrotoxicity.
Combination of two β -lactam antibiotics ^a	Avoids aminoglycoside nephrotoxicity. Potential antagonism. Resistance more likely to develop? Prolongation of neutropenia?
Single agents	
Cefoxitin (2nd-generation cephalosporin [cefamycin])	Substitute for aminoglycoside + ampicillin. No activity against <i>Pseudomonas</i> spp. Activity against anaerobes. More likely to disturb mucosal colonisation.
Ceftazidime (3rd-generation cephalosporin)	Less well-evaluated in veterinary medicine. Commonly used in human medicine for cancer patients. Expensive. Less activity against Gram-positive organisms than combination therapy.
Ceftiofur	Veterinary drug. Less well-evaluated than other treatments. Has been used for CPV-2 infection (Macintire, 1999).
Imipenem-cilastatin (carbapenem)	Commonly used in human medicine, and to a lesser extent in veterinary medicine, for cancer patients. Expensive. Has a broad antimicrobial spectrum.
Meropenem (carbapenem)	As per imipenem-cilastatin.

Notes: Doses are adapted from Allen (2004) and Greene (2005) and current use in the authors' practice; optimal doses in recommended dose ranges are not known. IV routes of administration are preferred, and all intravenous injections are given over 15-20 minutes unless indicated otherwise. *Aminoglycosides*: amikacin 15-20 mg/kg q24h, IV, IM, SC; gentamicin 5-6 mg/kg q24h, IV, IM, SC; netilmycin 6 mg/kg q24h, IV; tobramycin 6 mg/kg q24h, IV, IM, SC. In order to reduce the risks of nephrotoxicity due to aminoglycoside antibiotics, we use once daily administration, and avoid their use in dehydrated animals and in animals receiving furosemide. *Fluoroquinolones*: ciprofloxacin 5-10 mg/kg q12-24h, IV (1-hour infusion); enrofloxacin 5-10 mg/kg q12-24h IV, IM. The authors' initial dose is usually 5 mg/kg q12h IV. Higher doses are reserved for those cases where bacteria with higher MICs are suspected or isolated (e.g. *Pseudomonas* spp.). We do not recommend these drugs in cats. Enrofloxacin is approved for IM use only, but the solution is irritating to tissues and the authors prefer IV administration. For IV injection, the solution should be injected over 20-60 minutes; some recommend dilution of 1 part parenteral solution with 9 parts sterile water for injection. We do not recommend injecting the parenteral solution SC. Reduction in the frequency of administration and/or dose may be necessary in animals at risk for seizure activity (see text). *Aminobenzylpenicillins*: ampicillin 20-40 mg/kg q6-8h, IV, IM, SC; ampicillin-sulbactam 50 mg/kg q6-8h, IV, IM. *Anti-pseudomonal penicillins*: piperacillin 25-50 mg/kg q6-8h, IV, IM; piperacillin-tazobactam 25-50 mg/kg q6-8h, IV, IM; ticarcillin 40-75 mg/kg q6-8h, IV, IM; ticarcillin-clavulanate 30-50 mg/kg q6-8h IV, IM. *Cephalosporins*: cefazolin 20-30 mg/kg q6-8h, IV, IM, SC; cephalothin 25-40 mg/kg q6-8h, IV, IM, SC; cefoxitin 20-30 mg/kg q6-8h, IV, IM, SC; ceftazidime 25-30 mg/kg IV, IM, SC q8h (we usually dose these cephalosporins at 30 mg/kg q8h, IV); ceftiofur (dogs only) 2.2-4.4 mg/kg q12h, SC. *Carbapenems*: imipenem-cilastatin 2-10 mg/kg q6-8, IV (1-hour infusion), IM (reconstitute with 1% lidocaine without epinephrine). We usually use 5 mg/kg q8h IV. Meropenem (dogs only) 20 mg/kg q8h IV, 8 mg/kg q12h SC.

^aFor example, 1st-generation cephalosporin + anti-pseudomonal penicillin; 1st-generation cephalosporin + 3rd-generation cephalosporin; 3rd-generation cephalosporin + anti-pseudomonal penicillin.

of the possibility of inducing cartilage defects. However, the risks for such defects following three-to-five-day courses of treatment at standard doses is not known and its use in treating severe CPV-2 infection in pups has been recommended (Macintire, 1999). Fluoroquinolones may cause seizures and other neurologic signs at higher doses, especially with repetitive administration. Geriatric animals, animals with hypoalbuminemia, and animals with a history of seizures are at increased risk. Antibiotics may inhibit platelet function; this effect is most pronounced with penicillins in humans. Any such effects do not appear to be important in dogs (Wilkins et al., 1995; Webb et al., 2005) and are unlikely to be so in cats.

Reduction of fever is expected within 72 hours after starting antimicrobial therapy, and the animal should be more alert. Increasing depression coinciding with a falling temperature may be a sign of septic shock. In many cases, improvement is noted after the first dose. The duration of antibacterial therapy, once pyrexia has resolved, is controversial. Prolonged therapy increases expense, hospitalization, side effects, risk of selecting for resistant bacteria, and the risk of a fungal infection. Therapy should be continued for one to seven days beyond achievement of a neutrophil count of 0.5 to $1.0 \times 10^9/L$. Changing from IV therapy to oral therapy (Table 21.4) during this period facilitates discharge from the hospital and reduces expense. For cancer patients without a documented site of infection, we stop IV antimicrobial drugs the day after recovery of the neutrophil count to $1.0 \times 10^9/L$ and resolution of pyrexia. We continue oral antimicrobial prophylaxis in those patients that were receiving it, and give oral antimicrobials for seven days in those that were not. We do not recommend continuing with oral antimicrobials in patients recovering from parvoviral infections. In animals with pancytopenia with prolonged neutropenia, IV or oral antimicrobial therapy is continued for a minimum of 10 days beyond resolution of fever. At this time, withdrawal of antimicrobial therapy may be attempted.

Pyrexia may not resolve if: (1) it is not bacterial in origin (and this should be reconsidered); (2) the organism is not susceptible to the antimicrobial drug(s); (3) drug doses are too low; or (4) there is such a severe compromise of host defenses that the infection and associated fever will not respond to any antimicrobial agent. This last occurs with prolonged, severe neutropenia. This is infrequently encountered in veteri-

nary medicine, but has been observed during experimental marrow transplantation studies. Initial culture results may assist therapeutic decision making with unresponsive fever. If a resistant organism is documented, antimicrobial therapy may be changed based upon susceptibility testing. For animals with a bacterial pathogen that is susceptible in vitro but has not responded to empirical therapy, increasing the dose may result in clinical improvement. Once the animal is clinically stable, the medication may be continued until resolution of fever and achievement of a neutrophil count of $1.0 \times 10^9/L$. If there is a need to change the therapeutic regime, the choice of additional drugs depends on which antibiotics were used for initial therapy. Traditionally, failure of response to empirical therapy with cefoxitin or an aminoglycoside and first generation cephalosporin would prompt additional therapy against *Pseudomonas* spp. with an antipseudomonal penicillin. Ceftazidime, imipenem-cilastatin and meropenem could also be used to intensify activity against *Pseudomonas*. If a resistant Gram-negative organism is suspected (e.g., if there are signs of intestinal damage or respiratory signs), choices for additional therapy may include an aminoglycoside, fluoroquinolone, cefoxitin, ceftazidime, and other third generation cephalosporins, and imipenem-cilastatin or meropenem. Aztreonam may also be used in humans to intensify therapy against Gram-negative organisms and *Pseudomonas* spp., but there is limited veterinary experience with this drug.

Resistant Gram-positive organisms are increasingly responsible for infections in neutropenic humans, for which vancomycin and teicoplanin are the drugs of choice for empirical treatment. Veterinary experience with these drugs is also limited. If a resistant Gram-positive organism is suspected (e.g., if there are signs of phlebitis, injury to the skin or oral cavity, or respiratory signs), the drug of choice in animals is clindamycin, 10 mg/kg q12h , IV or SC, although it is bacteriostatic. Imipenem-cilastatin and meropenem could also be used, although activity against *Streptococcus* spp. may not be complete.

A non-responding fever may also be due to a resistant anaerobic infection. Additional therapy could include metronidazole ($15 \text{ mg/kg IV [1-hour infusion] q12h}$), clindamycin, cefoxitin, ampicillin-sulbactam, imipenem-cilastatin and meropenem. The latter two are suitable for increasing broad-spectrum antibacterial activity. Although imipenem-cilastatin and mero-

penem are expensive, they are less expensive than combined administration of an aminoglycoside or fluoroquinolone, first generation cephalosporin, and metronidazole, and in some cases are substituted for such combinations. If multiple antimicrobial agents are used, then selective withdrawal of agents may be considered once there is clinical improvement.

The preceding recommendations are appropriate for most cases, but may not be feasible due to cost restrictions and inability or unwillingness of the owner to return the animal to the hospital. In such cases, oral antimicrobial agents may be used if the animal is clinically stable. In addition, oral antimicrobial agents may be sufficient for initial treatment of neutropenic animals that have been febrile and clinically stable for several days. There is an increasing trend toward the use of oral antimicrobial therapy for the treatment of humans with low-risk febrile neutropenia, where the drugs of choice are ciprofloxacin and amoxicillin-clavulanate (Hughes et al., 2002; Rolston, 2004). For animals with mild neutropenia and mild pyrexia, therapy with trimethoprim-sulfonamide, a fluoroquinolone, amoxicillin, or amoxicillin-clavulanate is recommended. For animals with moderate to severe neutropenia or pyrexia, a fluoroquinolone plus cephalexin, amoxicillin or amoxicillin-clavulanate is recommended. Doses may be increased within standard recommendations (Allen, 2004; Greene, 2005) above those given in Table 21.4. Therapy with tetracyclines or doxycycline for ehrlichiosis may also control secondary infections. In all cases, the animal should be closely observed for clinical deterioration and arrangements made to initiate parenteral therapy. Oral antimicrobial therapy should not be used when the animal is hypovolemic, hypotensive, vomiting, or there is disruption of the intestinal mucosa.

With human neutropenic patients, if there is no response to multiple antibacterial agents after approximately 5 days of therapy, then empirical antifungal therapy may be initiated with amphotericin B, voriconazole, or caspofungin (Hughes et al., 2002; Rolston, 2004). This situation is not commonly encountered in veterinary medicine; antifungal therapy is not recommended in the dog or cat unless a fungal infection is documented. If neutropenia and antibacterial therapy persist beyond ten days, then stools should be monitored by culture or cytologic studies for candidal overgrowth and prophylaxis with nystatin, ketoconazole, or itraconazole considered, especially if antibac-

terial agents which disturb the mucosal bacterial flora (e.g., ampicillin, cefoxitin, metronidazole, imipenem-cilastatin, and meropenem) are being used.

Therapy of Documented Infections

An infection is considered documented in strict terms when the site of infection and infecting organism are both known. In broader terms, an infection is also considered documented if only the site of infection is known (e.g., radiographic evidence of pneumonia). Therapy of documented bacterial infections should consist of bactericidal antibiotics, with the choice based upon susceptibility testing. The guidelines for choosing parenteral or oral routes of administration are the same as those previously discussed. In most situations, by the time culture results are obtained, empirical therapy will have already been started.

The guidelines for duration of therapy with documented bacteremia but no localization into other organs are also as previously discussed. Treatment for documented pneumonia, as well as urinary tract and soft tissue infections, should be continued to a minimum of seven days beyond recovery of the neutrophil count to $1.0 \times 10^9/L$ and resolution of clinical and radiographic signs. The infection may transiently appear to become worse as neutrophil recovery occurs, due to increased inflammation. However, fever should be decreasing if the antimicrobial therapy is appropriate. The guidelines for intensifying therapy if fever and clinical signs are progressing are similar to those previously discussed, with drug selection aided by susceptibility test results.

Documented fungal infections should be treated with antimycotic drugs used at standard recommended doses (Allen, 2004; Greene, 2005). Amphotericin B is the current therapy of choice for infections caused by *Aspergillus* spp. Nephrotoxicity can be reduced by using the newer lipid-complex formulations, but the drugs are considerably more expensive. Some cases of topical and systemic aspergillosis can also be treated successfully with itraconazole. Amphotericin B is also indicated for treatment of systemic candidiasis, but therapy with ketoconazole or itraconazole may suffice (Weber et al., 1985). Intestinal candidiasis can be treated with nystatin, ketoconazole or itraconazole. Fluconazole is the drug of choice for urinary candidiasis. There is limited veterinary experience with the newer antifungal drugs voriconazole and caspofungin.

Bibliography

- Abrams-Ogg ACG, et al. 1993. Clinical and pathologic findings in dogs following supralethal total body irradiation with and without infusion of autologous long-term marrow culture cells. *Can J Vet Res* 57:79.
- Allen DG. 2004. *Handbook of Veterinary Drugs*. 3rd ed. Baltimore: Lippincott Williams & Wilkins.
- Bodey GP, et al. 1966. Quantitative relationship between circulating leukocytes and infections in patients with acute leukemia. *Ann Int Med* 64:328.
- Brown J. 2005. Zygomycosis: An emerging fungal infection. *Am J Health Syst Pharm* 62:2593.
- Brown RM, Rogers KS. 2001. Neutropenia in dogs and cats. *Comp Cont Ed Pract Vet* 23:534.
- Couto CG. 1990. Management of complications of cancer chemotherapy. *Vet Clin North Am Small Anim Pract* 20:1037.
- Crawford J, et al. 2004. Chemotherapy-induced neutropenia: risks, consequences, and new directions for its management. *Cancer* 100:228.
- Dahlinger J, et al. 1997. Prevalence and identity of translocating bacteria in healthy dogs. *J Vet Intern Med* 11:319.
- De Pauw B. 2004. Preventative use of antifungal drugs in patients treated for cancer. *J Antimicrob Chemother* 53:130.
- Donnelly JP. 1997. Is there a rationale for the use of antimicrobial prophylaxis in neutropenic patients? *J Intern Med* 242(Suppl 740):79.
- Ehrensaft DV, et al. 1979. Disseminated candidiasis in leukopenic dogs. *Proc Soc Exp Biol Med* 160:6.
- Ellis M. 2004. Preventing microbial translocation in haematological malignancy. *Br J Haematol* 125:282.
- Feld R. 1989. The compromised host. *Eur J Cancer Clin Oncol* 25(Suppl 2):S1.
- Fox JG, et al. 1978. Systemic fungal infections in cats. *J Am Vet Med Assoc* 173:1191.
- Glasmacher A, et al. 1996. Antifungal prophylaxis with itraconazole in neutropenic patients: Pharmacological, microbiological and clinical aspects. *Mycoses* 39:249.
- Gorschlüter M, et al. 2001. *Clostridium difficile* infection in patients with neutropenia. *Clin Infect Dis* 33:786.
- Greene CE, ed. 2005. *Infectious Diseases of the Dog and Cat*. 3rd ed. Philadelphia: Elsevier (Saunders).
- Greenfield CL, et al. 2000. Results of hematologic analyses and prevalence of physiologic leukopenia in Belgian Tervuren. *J Am Vet Med Assoc* 216:866.
- Hammon WD, Enders JE. 1939. A virus disease of cats, principally characterized by a leucocytosis, enteric lesions and the presence of intranuclear inclusion bodies. *J Exp Med* 67:327.
- Holzworth J. 1987. Mycotic diseases. In: Holzworth J, ed. *Diseases of the Cat: Medicine and Surgery*. Philadelphia: WB Saunders.
- Hughes WT, et al. 2002. 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer. *Clin Infect Dis* 34:730.
- Jacobs G, et al. 1998. Neutropenia and thrombocytopenia in three dogs treated with anticonvulsants. *J Am Vet Med Assoc* 212:681.
- Klastersky J. 1989. Infections in compromised hosts: considerations on prevention. *Eur J Cancer Clin Oncol* 25(Suppl 2):S53.
- Klastersky J. 1997. Treatment of neutropenic infection: Trend towards monotherapy? *Support Care Cancer* 5:365-370.
- Koutinas AF, et al. 1998. Asymptomatic bacteriuria in puppies with canine parvovirus infection: a cohort study. *Vet Microbiol* 63:109.
- Kunkle GA, et al. 1995. Adverse effects of oral antibacterial therapy in dogs and cats: an epidemiologic study of pet owners' observations. *J Am Anim Hosp Assoc* 31:46.
- Lobetti RG, et al. 2002. Bacterial colonization of intravenous catheters in young dogs suspected to have parvoviral enteritis. *J Am Vet Med Assoc* 220:1321.
- Macintire DK. 1999. Pediatric intensive care. *Vet Clin North Am Small Anim Pract* 29:971.
- Neth OW, et al. 2005. Susceptibility to infection in patients with neutropenia: the role of the innate immune system. *Br J Haematol* 129:713.
- Ochiai K, et al. 2000. Intestinal candidiasis in a dog. *Vet Rec* 146:228.
- Picazo JJ. 2004. Management of the febrile neutropenic patient: a consensus conference. *Clin Infect Dis* 39 Suppl 1:S1.
- Reller LB. 1994. What the practicing physician should know about blood cultures. In: Koontz F, ed. *Blood Culture Controversies—Revisited*. Iowa City, IA: American Society of Microbiology.
- Rodriguez F, et al. 1998. Acute disseminated candidiasis in a puppy associated with parvoviral infection. *Vet Rec* 142:434.
- Rolston KV. 2004. Management of infections in the neutropenic patient. *Annu Rev Med* 55:519.
- Rosenthal RC. 1988. Autologous bone marrow transplantation for lymphoma. *Proc 6th Annu Vet Med Forum Am Coll Vet Int Med* 397.
- Scott FW. 1987. Viral diseases. In: Holzworth J, ed. *Diseases of the Cat*. Philadelphia: WB Saunders.
- Sipsas NV, et al. 2005. Perspectives for the management of febrile neutropenic patients with cancer in the 21st century. *Cancer* 103:1103.
- Trepanier LA. 2004. Idiosyncratic toxicity associated with potentiated sulfonamides in the dog. *J Vet Pharmacol Ther* 27:129.
- Turk J, et al. 1990. Coliform septicemia and pulmonary disease associated with canine parvoviral enteritis: 88 cases (1987-1988). *J Am Vet Med Assoc* 196:771.
- Turk J, et al. 1992. Enteric *Clostridium perfringens* infection associated with parvoviral enteritis in dogs: 74 cases (1987-1990). *J Am Vet Med Assoc* 200:991.
- Van Der Waaij D. 1988. Selective decontamination of the digestive tract: General principles. *Eur J Cancer Clin Oncol* 24(Suppl 1):S1.
- van de Wetering MD, et al. 2005. Efficacy of oral prophylactic antibiotics in neutropenic afebrile oncology patients: a systematic review of randomised controlled trials. *Eur J Cancer* 41:1372.
- Veterinary Co-Operative Oncology Group. 2004. Veterinary co-operative oncology group—common terminology cri-

- teria for adverse events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats v1.0. *Vet Comp Oncology* 2:194.
- Wade JC. 1994. Epidemiology and prevention of infection in the compromised host. In: Rubin RH, Young LS, eds. *Clinical Approach to Infection in the Compromised Host*. 3rd ed. New York: Plenum.
- Warren AL, et al. 2001. Multi-drug resistant *Escherichia coli* with extended-spectrum β -lactamase activity and fluoroquinolone resistance isolated from clinical infections in dogs. *Aus Vet J* 79:621.
- Webb JA, et al. 2005. Effect of antimicrobials on coagulation parameters in healthy dogs. *J Vet Intern Med* 19:447.
- Weber MJ, et al. 1985. Treatment of systemic candidiasis in neutropenic dogs with ketoconazole. *Exp Hematol* 13:791.
- Weese JS, Armstrong J. 2003. Outbreak of *Clostridium difficile*-associated disease in a small animal veterinary teaching hospital. *J Vet Intern Med* 17:813.
- Weiss DJ. 1995. Leukocyte disorders and their treatment. In: Bonagura JD, ed. *Kirk's Current Veterinary Therapy XII: Small Animal Practice*. Philadelphia: WB Saunders.
- Wilkens B, et al. 1995. Effects of cephalothin, cefazolin, and cefmetazole on the hemostatic mechanism in normal dogs: implications for the surgical patient. *Vet Surg* 24:25.
- Williamson NL, et al. 2002. Effects of short-term trimethoprim-sulfamethoxazole administration on thyroid function in dogs. *J Am Vet Med Assoc* 221:802.
- Wilson BJ. 2002. Dietary recommendations for neutropenic patients. *Semin Oncol Nurs* 18:44.

Antimicrobial Therapy of Selected Organ Systems

Patricia M. Dowling, Stephen A. Kruth

Osteomyelitis, Septic Arthritis and Septic Tenosynovitis

Patricia M. Dowling

Because of the variety of pathogens involved in musculoskeletal infections, appropriate samples must be submitted for microbiological culture and susceptibility testing. Because of the devastating consequences of bone, joint, or tendon sheath infections, aggressive antimicrobial therapy must be initiated as soon as there is sufficient evidence of infection. While awaiting culture results, initial treatment can be selected based on the clinical case characteristics and retrospective studies.

Osteomyelitis

Osteomyelitis is acute or chronic inflammation of the bone and its structures secondary to infection with pyogenic organisms. The infection can be limited to a single portion of bone or can involve several regions, such as the marrow, cortex, periosteum, surrounding soft tissue, or synovial structures at the ends of the bone. Osteomyelitis can be hematogenous, traumatic, or iatrogenic in origin. Hematogenous infections are almost exclusively seen in septic neonates and typically occur in a joint, epiphysis, or physis. In young animals, the endothelium of capillaries in the epiphysis is discontinuous, which allows extravasation of bacteria. Blood leukocytes are absent from this area, so tissue macrophages are the sole defense against bacterial invasion. The incompetence of these tissue macrophages appears critical to development of hematogenous metaphyseal osteomyelitis in young animals. Traumatic infections are usually secondary to a laceration or puncture wound and can infect bone, joint, tendon sheath, or bursa, and often involve multiple organ-

isms. Iatrogenic infections are usually secondary to surgical procedures, with or without implants. Osteomyelitis associated with implants presents the greatest treatment challenge, so broad-spectrum prophylactic antimicrobial therapy is indicated with any procedure that requires surgical implants (Chapter 21).

Microbial and host factors are both involved in the development of osteomyelitis. The bacteria involved in osteomyelitis have a range of extracellular and cell-associated factors that contribute to their virulence. Bacterial adhesins promote attachment to extracellular matrix proteins, which is crucial for colonization of host tissues and implanted biomaterials. *Staphylococcus aureus* expresses several adhesins on its surface, each specifically interacting with one host protein component, such as fibrinogen, fibronectin, collagen, vitronectin, laminin, thrombospondin, bone sialoprotein, elastin, or von Willebrand factor.

Other bacterial factors promote evasion from host defenses (protein A, some toxins, capsular polysaccharides). A third set promotes invasion or tissue penetration by specifically attacking host cells (exotoxins) or degrading components of extracellular matrix (various hydrolases).

Some pathogens involved in osteomyelitis produce biofilm, populations of bacteria that attach to a surface or to each other and embedded in a matrix of extracellular polymeric substance. Biofilm bacteria show altered phenotypes in terms of growth, gene expression, and protein production. Biofilms act as a diffusion barrier, slowing down the penetration of antimicrobials.

Chronic osteomyelitis is characterised by infected necrotic bone and poor local vascularization within a compromised soft-tissue envelope. Systemic symptoms generally subside, but one or more foci in the bone still contain infected tissue or a sequestrum. The

infected foci are surrounded by sclerotic, avascular bone covered by a thickened periosteum and scarred muscle and subcutaneous tissue. This avascular envelope makes systemic antimicrobials essentially ineffective. Intermittent exacerbations can occur for years and only respond temporarily to antimicrobials.

Identification of the causative microorganisms is essential for diagnosis and treatment of osteomyelitis. Surgical sampling or needle biopsies of infected tissue are the best methods of diagnosis, as culture from swabs of ulcers or fistulae is often misleading. Sometimes only the histopathological examination of a bone-biopsy specimen with special staining procedures provides an accurate diagnosis of the infection (Lew and Waldvogel, 2004).

In a retrospective study of bacterial culture and susceptibility results from 233 horses and foals with musculoskeletal infections, 91% of the bacteria were aerobic or facultative and 9% were anaerobic (Moore et al., 1992). The common bacteria isolated included enterobacteriaceae (29%), non-beta-hemolytic streptococci (13%), coagulase-positive staphylococci (12%), beta hemolytic streptococci (9.4%), and coagulase-negative staphylococci (7.3%). Enterobacteriaceae are the most common bacteria associated with osteomyelitis in septic foals and calves. *Salmonella dublin* has been isolated from lesions of septic calves (Healy et al., 1997). *Arcanobacterium pyogenes* is the most common causative agent of osteomyelitis in adult cattle (Verschooten et al., 2000). *Actinomyces bovis* causes mandibular pyogranulomatous osteomyelitis ("lumpy jaw") in ruminants (Seifi et al., 2003). Infectious pododermatitis ("foot rot") can progress to osteomyelitis, usually *A. pyogenes* and *Fusobacterium necrophorum* are involved (Silva et al., 2004). Osteomyelitis in dogs and cats is most commonly caused by *Staphylococcus intermedius*, but there are reports of *S. aureus* infections (Johnson, 1994; Rahal et al., 2003). Polymicrobial infections are common in small animals, and may include mixtures of streptococci, enterococci, enterobacteriaceae (*E. coli*, *Klebsiella* spp.), and anaerobic bacteria.

Septic Arthritis and Tenosynovitis

Septic arthritis is inflammation of the joint space caused by a variety of opportunist pathogens that reach the joint hematogenously, by puncture, or by extension from adjacent infected tissues. The normal joint can withstand a large bacterial challenge, but with sufficient virulence and pathogenicity, the syn-

ovial defenses are overcome and infection is successfully established. With colonization of the synovium, a variety of enzymes, free radicals, and other inflammatory mediators initiate a marked synovial inflammatory response.

Culture of synovial fluid is more diagnostic than culture of the synovial membrane. Synovial membrane biopsy can increase the chance of a positive culture result, but positive cultures are obtained from only 75% of cases (Schneider et al., 1992). Septic arthritis from Gram-negative bacteria (*E. coli*, *Salmonella*, *Pseudomonas*, *Klebsiella*, etc.) is common in large animal neonates with failure of transfer of passive immunity. Involvement of more than one joint occurs in more than 50% of foals with septic arthritis; multiple joint involvement is uncommon in adult horses (Schneider et al., 1992a). In adult animals, septic arthritis and tenosynovitis commonly result from wounds or iatrogenic contamination with bacteria. In wounds, a variety of Gram-positive and Gram-negative bacteria are typical, whereas *S. aureus* and *S. intermedius* are the usual isolates from iatrogenic infections. Methicillin-resistant *S. aureus* has been reported in a case of septic arthritis in a dog (Owen et al., 2004).

It is important that septic arthritis and tenosynovitis be treated as soon as possible to prevent articular cartilage destruction, tendon sheath adhesions and degenerative joint disease. A precise microbiological diagnosis is critical, but treatment can be started on the basis of Gram stain from joint or tendon sheath aspirates while awaiting culture results. Drainage of the joint or tendon sheath is essential to remove bacteria, debris, and inflammatory products that cause cartilage damage and adhesions, as well as to reduce intra-articular pressure that may cause ischemic necrosis (Bubenik, 2005). Repeated closed-needle aspiration (every 12 or 24 hours for 7–10 days) can be done in some veterinary patients. Joint aspiration may be adequate in early stages of septic arthritis, but repeated distension irrigation or joint lavage is recommended if clinical improvement does not occur within 24–48 hours.

Systemic Antimicrobial Therapy

In acute osteomyelitis, parenteral treatment should start as soon as culture specimens are taken and be administered in high doses for a minimum of 3 weeks. If necessary, the initial antimicrobial agent can be replaced depending on susceptibility test results. Oral

antibiotic treatment is then continued for an additional 3 weeks. Bactericidal antimicrobials are required. Antimicrobial therapy alone is adequate for the treatment of most cases of acute osteomyelitis.

Chronic osteomyelitis is treated by surgical removal of sequestra, often following 14–21 days of parenteral antimicrobial therapy. Ideally, parenteral antimicrobials should be administered after surgery and replaced after 3–4 weeks by oral antimicrobials, which should be continued for a further 1–2 months. Treatment of chronic osteomyelitis requires aggressive and prolonged treatment which achieves adequate, pharmacodynamically appropriate, local concentrations of bactericidal drugs.

Apart from antimicrobial therapy, the cornerstones of osteomyelitis treatment include debridement and sequestrectomy, open wound drainage, fracture stabilization, and grafting of bone deficits (Johnson, 1994; Lew and Waldvogel, 2004). Thorough debridement of bone and soft tissue to remove necrotic debris, purulent material, and avascular bone is imperative for treatment success. Wound debridement should be combined with appropriate stabilization of unstable fractures and mineralization or removal of metallic implants. Stable fractures can heal in the face of infection. At the time of debridement, affected tissue should be obtained for culture and sensitivity to assist the clinician in choosing the most appropriate antimicrobial drug.

Antimicrobial therapy should be with bactericidal drugs, ideally administered parenterally for 2 weeks with subsequent oral administration for a further 4–6 weeks. Most antimicrobials traverse the capillary membrane in normal and infected bone, and concentrations in bone closely parallel plasma concentrations. Vascular thrombosis and ischemia of infected bone and synovium can limit the delivery of systemic antimicrobials in sufficient concentrations to eradicate the infection.

For most bone and joint infections caused by beta-lactamase producing staphylococci, cephalosporins (cefazolin, cephalexin, ceftiofur), clindamycin, or amoxicillin-clavulanate will be effective. Newer human macrolide antimicrobials (azithromycin, clarithromycin) may also be efficacious. In small animals, clindamycin and metronidazole are used for anaerobic infections. The aminoglycosides and fluoroquinolones also typically have good activity against staphylococci, along with excellent activity against Gram-negative

pathogens. While amikacin usually has good activity against *Pseudomonas* spp., it has poor activity against streptococci compared to gentamicin. Because of nephrotoxicity and ototoxicity related to duration of treatment, the aminoglycosides are often reserved for treatment of musculoskeletal infections by local delivery techniques. The fluoroquinolones' safety and excellent broad-spectrum antimicrobial activity, and their availability in injectable and oral formulations, make them popular choices for treatment of musculoskeletal infections in many veterinary patients.

Local Antimicrobial Drug Delivery

Antimicrobial drug delivery systems (DDS) have been developed for use in human and veterinary patients, providing sustained high local drug concentrations while minimizing systemic toxicity. An antimicrobial DDS can achieve high drug concentrations at the site of infection while maintaining low systemic drug levels and avoiding possible adverse effects (Wang et al., 2002). Methods of local administration of antimicrobials include biodegradable and non-biodegradable implants, constant rate infusion or indwelling catheter systems, local injection, and regional limb perfusion either by intravenous or intraosseous routes.

Non-biodegradable Antimicrobial Impregnated Implants

Polymethyl methacrylate (PMMA) is a synthetic polymer marketed in powder form in North America. Antimicrobials in powder form can be added to the polymer to make non-biodegradable antimicrobial impregnated implants for the treatment of osteomyelitis and septic arthritis (Holcombe et al., 1997; Tobias et al., 1996). In Europe, PMMA is available in combination with gentamicin in pre-made beads (Septopal®). The antimicrobials used to make PMMA beads must be heat stable, as the combination of the liquid monomer and the powder polymer produces an exothermic reaction. The antimicrobial must have adequate elution characteristics to produce a sustained and appropriate release from the bead.

Antimicrobial elution from the PMMA bead depends upon the pore size, permeability, size, and shape of the implant, and the type and amount of antimicrobial present in the bead (Weisman et al., 2000). The amount and rate of wound exudation also affects the elution kinetics of the antimicrobial from the bead (Streppa et al., 2001). Release of an antimicrobial from PMMA is bimodal. There is a rapid release during the

first 24 hours after implantation, followed by a continuous sustained release that can last from weeks to years (Calhoun and Mader, 1989).

Osteomyelitis in horses, cattle, dogs, and exotic animals has been successfully treated using PMMA beads (Butson et al., 1996; Hartley and Sanderson, 2003; Trostle et al., 1996; Trostle et al., 2001). Due to potential synovial irritation and lameness, PMMA use is not recommended inside joints (Farnsworth et al., 2001). Perhaps the most negative aspect of their use is their non-biodegradable nature. Although most tissues do not seem to react to the presence of the beads, tissue irritation is possible and in these cases implant removal is recommended.

Biodegradable Antimicrobial Impregnated Implants

Various biodegradable DDS such as collagen sponges, hydroxyapatite cement, plaster of Paris, polyanhydrides, polylactide-polyglycolide, and crosslinked high amylose starch have been explored for use as DDS. Their major advantage over PMMA is that a second surgery for removal is not necessary. Currently, their cost/benefit ratio is often too high to permit their use in veterinary medicine.

Collagen Sponges

Commercially available gentamicin-impregnated collagen sponges are available in Europe but not in North America. Their clinical use has been reported in cattle, horses, and dogs (Hirsbrunner and Steiner, 1998; Owen et al., 2004; Summerhays, 2000; Zulauf M et al., 2001). In contrast to PMMA beads, complete elution from collagen sponges occurs in a period of two weeks, with high elution rates during the first week. The main disadvantages of gentamicin-impregnated collagen are its expense and the potential for adverse reactions to a foreign protein, as its source is bovine collagen.

Hydroxyapatite Cement

Hydroxyapatite cement (HAC) implants are made from marine coral, which allows vascular and fibrous tissue growth from the host into the implant. The HAC is fabricated with water, which can be replaced with a liquid antimicrobial formulation. In an in vitro study, gentamicin, amikacin or ceftiofur eluted at greater concentrations from HAC than from PMMA, but both gentamicin and amikacin released bactericidal concentrations of antibiotic for at least 30 days, while ceftiofur concentrations dropped precipitously

after 2 weeks (Ethell et al., 2000). Although HAC appears to have optimal qualities for a biodegradable delivery system, additional in vivo studies are necessary to determine its efficacy and safety.

Plaster of Paris

Plaster of Paris (POP) is an inexpensive, readily available material that has been investigated for use as an antimicrobial DDS. Plaster of Paris gentamicin-impregnated beads are inexpensive, biocompatible, biodegradable, osteoconductive and easily manufactured using liquid antimicrobials and a bead mold (Dahners and Funderburk, 1987; Santschi and McGarvey, 2003). The relatively short duration of high concentrations of gentamicin eluted from POP beads suggest that they may be ideal for antimicrobial prophylaxis in high-risk situations such as fracture repair.

Polyanhydrides

Polyanhydrides are a class of biodegradable polymers that have a hydrophobic backbone with hydrolytically labile anhydride linkages, such that hydrolytic degradation can be controlled by manipulation of the polymer composition. They degrade in vitro and in vivo to their acid counterparts, and do not cause an inflammatory reaction. The main limitation of polyanhydrides is their storage stability—they require refrigeration. A polyanhydride/gentamicin release system has been used for the treatment of soft tissue infections, osteomyelitis and septic arthritis in rats, horses, and humans (Laurencin et al., 1993; Li et al., 2002).

Polylactide-Polyglycolide

An in vitro study in equine synovium comparing poly[D,L]-lactide and poly[D,L]-lactide-co-glycolide showed equivalent controlled release of gentamicin at high concentrations for ten days, and elimination of infection with no adverse effects on synovial HA production or viability (Cook et al., 1999). The release was biphasic, consisting of a slow induction period and then a period of more rapid release. The latter consisted of a spiked release of gentamicin in the first 24 hours, followed by sustained release for 10 days, after which release was <10 µg/mL through day 14.

Crosslinked High Amylose Starch

Crosslinked high amylose starch (CLHAS) is a biodegradable material characterized by excellent biocompatibility and controlled local antimicrobial delivery

properties after SC and IM implantations. Moreover, CLHAS implants are easily manufactured, which is an advantage over most other biodegradable DDS. Following placement of ciprofloxacin-loaded CLHAS implants in rabbits, local muscle and bone concentrations were over 100 times in excess of the MIC for *S. aureus* for at least 28 days (Desevaux et al., 2002). Neovascularization and fibrous septae eventually subdivide CLHAS implants, which are then progressively phagocytosed by macrophages. In a dog model of osteomyelitis, oral ciprofloxacin and ciprofloxacin-CLHAS implants had equal efficacy (Huneault et al., 2004).

Constant Rate Infusion or Indwelling Systems

Constant delivery of antimicrobials is indicated for infections of synovial cavities such as joints or tendon sheaths. The use of commercial constant rate infusion pumps or "in-house" manufactured delivery systems has been reported in horses (Lescun et al., 2000; Lescun et al., 2002). Affected structures treated with such systems include the distal interphalangeal, metacarpo/tarso-phalangeal, intercarpal, radiocarpal, scapulohumeral, tarsocrural, and medial femoropatellar joints, the carpal canal, and the digital, tarsal, and extensor carpi radialis tendon sheaths. Horses tolerate the tubing well with no apparent discomfort and with only mild soft-tissue swelling as a complication. This method allows frequent administration of high concentrations of the appropriate antimicrobial to the infected site. Daily joint lavage can be accomplished through the same system.

Intra-articular Injections

Intra-articular or intra-synovial injection of antimicrobial drugs achieves high synovial fluid and bone concentrations with low doses (Werner et al., 2003). Gentamicin, amikacin, ceftiofur and cefazolin are used, with minimal inflammatory effects on the synovium (Mills et al., 2000; Schneider et al., 1992b). Intra-articular or intra-synovial antimicrobials are usually infused after daily through and through lavage. As intra-articular or intra-synovial injection does not produce similar high concentrations in the surrounding soft tissues, systemic antimicrobials are used concurrently.

Regional Perfusion

Regional limb perfusion (RLP) techniques are used predominantly in large animals to deliver very high

antimicrobial concentrations in the distal extremities using the venous system, which is isolated from the systemic circulation by the controlled application of a tourniquet. Pressurizing the venous system allows diffusion of antimicrobials into ischemic tissues and exudates. The RLP techniques are limited to distal extremity areas, as it is impossible to isolate regions of the proximal extremity successfully. Thus areas above the mid-radius or mid-tibia are not good candidates for this technique.

Regional limb perfusion can be done by the intravenous or intraosseous route and is easily done in the standing, sedated horse. Intravenous RLP is performed by catheterizing the cephalic, saphenous, palmar metacarpal, or plantar metatarsal vein (Murphey et al., 1999; Navarre et al., 1999). However, any visible and accessible vein can be used safely to administer antimicrobials.

Alternatively, an antimicrobial solution can be administered by the intraosseous route by infusion into the medullary cavity of the cannon bone, tibia, or radius (Butt et al., 2001; Mattson S, et al. 2004). While both RLP techniques are efficacious, many horses and cattle with septic arthritis and tenosynovitis following trauma have generalized cellulitis of the limb, making localization of a superficial vein extremely difficult. If a superficial vein is localized, its structure is often disrupted by venipuncture, making repeated catheterization difficult. In addition, digital IV catheters are difficult to maintain in large animal patients. The intraosseous perfusion technique eliminates the need to find a vein and the trauma of repeated venipuncture or catheterization of distal veins. It enables repeated local perfusion with relative ease.

There are many unanswered questions regarding the appropriate choice and dose of antimicrobial, the best perfusion volume, the optimal number of perfusions, and the appropriate interval between perfusions for horses or cattle with septic conditions in a distal limb. Currently, it is recommended that the distal limb be perfused once daily for a total of 4 days.

Antimicrobials currently used for RLP in horses and cattle include amikacin, gentamicin, cefazolin, ceftiofur, sodium penicillin G, and vancomycin (Butt et al., 2001; Gagnon et al., 1994; Murphey et al., 1999; Navarre et al., 1999; Pille, et al. 2005; Rubio-Martinez et al., 2005a; Rubio-Martinez et al., 2005b).

Bibliography

- Bubenik LJ. 2005. Infections of the skeletal system. *Vet Clin North Am Small Anim Pract* 35:1093.
- Butson RJ, et al. 1996. Treatment of intrasynovial infection with gentamicin-impregnated polymethylmethacrylate beads. *Vet Rec* 138:460.
- Butt TD, et al. 2001. Comparison of 2 techniques for regional antibiotic delivery to the equine forelimb: intraosseous perfusion vs. intravenous perfusion. *Can Vet J* 42:617.
- Calhoun JH, Mader JT. 1989. Antibiotic beads in the management of surgical infections. *Am J Surg* 157:443.
- Cook VL, et al. 1999. Biodegradable drug delivery systems for gentamicin release and treatment of synovial membrane infection. *Vet Surg* 28:233.
- Dahners LE, Funderburk CH. 1987. Gentamicin-loaded plaster of Paris as a treatment of experimental osteomyelitis in rabbits. *Clin Orthop Relat Res*(219):278.
- Desevaux C, et al. 2002. Characterization of crosslinked high amylose starch matrix implants. 2. In vivo release of ciprofloxacin. *J Control Release* 82:95.
- Ethell MT, et al. 2000. In vitro elution of gentamicin, amikacin, and ceftiofur from polymethylmethacrylate and hydroxyapatite cement. *Vet Surg* 29:375.
- Farnsworth KD, et al. 2001. The effect of implanting gentamicin-impregnated polymethylmethacrylate beads in the tarsocrural joint of the horse. *Vet Surg* 30:126.
- Gagnon H, et al. 1994. Single-dose pharmacokinetics of ceftazolin in bovine synovial fluid after intravenous regional injection. *J Vet Pharmacol Ther* 17:31.
- Johnson KA. 1994. Osteomyelitis in dogs and cats. *J Am Vet Med Assoc* 205:1882.
- Hartley MP, Sanderson S. 2003. Use of antibiotic impregnated polymethylmethacrylate beads for the treatment of chronic mandibular osteomyelitis in a Bennett's wallaby (*Macropus rufogriseus rufogriseus*). *Aust Vet J* 81:742.
- Healy AM, et al. 1997. Cervico-thoracic vertebral osteomyelitis in 14 calves. *Vet J* 154:227.
- Hirsbrunner G, Steiner A. 1998. Treatment of infectious arthritis of the radiocarpal joint of cattle with gentamicin-impregnated collagen sponges. *Vet Rec* 142:399.
- Holcombe SJ, et al. 1997. Use of antibiotic-impregnated polymethyl methacrylate in horses with open or infected fractures or joints: 19 cases (1987–1995). *J Am Vet Med Assoc* 211:889.
- Huneault LM, et al. 2004. Prevention and treatment of experimental osteomyelitis in dogs with ciprofloxacin-loaded crosslinked high amylose starch implants. *J Orthop Res* 22:1351.
- Laurencin CT, et al. 1993. Bioerodible polyanhydrides for antibiotic drug delivery: in vivo osteomyelitis treatment in a rat model system. *J Orthop Res* 11:256.
- Lescun TB, et al. 2000. Continuous infusion of gentamicin into the tarsocrural joint of horses. *Am J Vet Res* 61:407.
- Lescun TB, et al. 2002. Effects of continuous intra-articular infusion of gentamicin on synovial membrane and articular cartilage in the tarsocrural joint of horses. *Am J Vet Res* 63:683.
- Lew DP, Waldvogel FA. 2004. Osteomyelitis. *Lancet* 364:369.
- Li LC, et al. 2002. Polyanhydride implant for antibiotic delivery—from the bench to the clinic. *Adv Drug Deliv Rev* 4:963.
- Mattson S, et al. 2004. Intraosseous gentamicin perfusion of the distal metacarpus in standing horses. *Vet Surg* 33:180.
- Mills ML, et al. 2000. Determination of synovial fluid and serum concentrations, and morphologic effects of intraarticular ceftiofur sodium in horses. *Vet Surg* 29:398.
- Moore RM, et al. 1992. Antimicrobial susceptibility of bacterial isolates from 233 horses with musculoskeletal infection during 1979–1989. *Equine Vet J* 24:450.
- Murphey ED, et al. 1999. Regional intravenous perfusion of the distal limb of horses with amikacin sulfate. *J Vet Pharmacol Ther* 22:68.
- Navarre CB, et al. 1999. Ceftiofur distribution in plasma and joint fluid following regional limb injection in cattle. *J Vet Pharmacol Ther* 22:13.
- Owen MR, et al. 2004. Management of MRSA septic arthritis in a dog using a gentamicin-impregnated collagen sponge. *J Small Anim Pract* 45:609.
- Pille F, et al. 2005. Synovial fluid and plasma concentrations of ceftiofur after regional intravenous perfusion in the horse. *Vet Surg* 34(6):610.
- Rahal SC, et al. 2003. Osteomyelitis associated with an orthopedic implant. *Can Vet J* 44:597.
- Rubio-Martinez L, et al. 2005a. Medullary plasma pharmacokinetics of vancomycin after intravenous and intraosseous perfusion of the proximal phalanx in horses. *Vet Surg* 34:618.
- Rubio-Martinez LM, et al. 2005b. Evaluation of safety and pharmacokinetics of vancomycin after intravenous regional limb perfusion in horses. *Am J Vet Res* 66:2107.
- Santschi EM, McGarvey L. 2003. In vitro elution of gentamicin from Plaster of Paris beads. *Vet Surg* 32:128.
- Schneider RK, et al. 1992a. A retrospective study of 192 horses affected with septic arthritis/tenosynovitis. *Equine Vet J* 24:436.
- Schneider RK, et al. 1992b. Open drainage, intra-articular and systemic antibiotics in the treatment of septic arthritis/tenosynovitis in horses. *Equine Vet J* 24:443.
- Seifi HA, et al. 2003. Mandibular pyogranulomatous osteomyelitis in a Sannen goat. *J Vet Med A Physiol Pathol Clin Med* 50:219.
- Silva LA, et al. 2004. Comparative study of three surgical treatments for two forms of the clinical presentation of bovine pododermatitis. *Ann N Y Acad Sci* 1026:118.
- Streppa HK, et al. 2001. Applications of local antimicrobial delivery systems in veterinary medicine. *J Am Vet Med Assoc* 219:40.
- Summerhays GE. 2000. Treatment of traumatically induced synovial sepsis in horses with gentamicin-impregnated collagen sponges. *Vet Rec* 147:184.
- Tobias KMS, et al. 1996. Use of antimicrobial-impregnated polymethyl methacrylate. *J Am Vet Med Assoc* 208:841.
- Trostle SS, et al. 1996. Use of antimicrobial-impregnated polymethyl methacrylate beads for treatment of chronic, refractory septic arthritis and osteomyelitis of the digit in a bull. *J Am vet Med Assoc* 208:404.

- Trostle SS, et al. 2001. Treatment of methicillin-resistant *Staphylococcus epidermidis* infection following repair of an ulnar fracture and humeroradial joint luxation in a horse. *J Am Vet Med Assoc* 218:554.
- Verschooten F, et al. 2000. Bone infection in the bovine appendicular skeleton: a clinical, radiographic, and experimental study. *Vet Radiol Ultrasound* 41:250.
- Wang J, et al. 2002. The application of bioimplants in the management of chronic osteomyelitis. *Orthopedics* 25:1247.
- Weisman DL, et al. 2000. In vitro evaluation of antibiotic elution from polymethylmethacrylate (PMMA) and mechanical assessment of antibiotic-PMMA composites. *Vet Surg* 29:245.
- Werner LA, et al. 2003. Bone gentamicin concentration after intra-articular injection or regional intravenous perfusion in the horse. *Vet Surg* 32:559.
- Zulauf M, et al. 2001. Fenestration of the abaxial hoof wall and implantation of gentamicin-impregnated collagen sponges for the treatment of septic arthritis of the distal interphalangeal joint in cattle. *Vet Rec* 149:516.

Infections of the Eyes: Conjunctivitis, Keratitis, and Endophthalmitis

Patricia M. Dowling

While the eye is relatively impermeable to microorganisms, if structural damage occurs, sight-threatening bacterial and fungal infections can easily develop. Ocular antimicrobial therapy differs from treating infections in other tissues because drugs can be administered directly to the eye, achieving high drug concentrations. However, there are only a limited number of veterinary-approved antimicrobials for topical ophthalmic use, so practitioners need to make rational antimicrobial choices and extra-label drug use is sometimes necessary for successful therapy. Practitioners should avoid using antimicrobials to treat noninfectious ocular conditions such as uveitis or allergic conjunctivitis. Unwarranted antimicrobials have no effect on an inflammatory disease process and encourage antimicrobial resistance. If a clear decision cannot be made between using an antimicrobial or an anti-inflammatory drug, consult a veterinary ophthalmologist.

Culture and Susceptibility Testing of Ocular Pathogens

As with an infection in any other tissue, tentative identification of the pathogen(s) involved in ocular infections is essential in choosing appropriate antimicrobial therapy. A Gram stain of corneal ulcer scrapings may

be used to initially identify pathogens as Gram-positive or Gram-negative bacteria, or as fungi. The immediate information gained from cytology is invaluable in directing initial antimicrobial therapy (Massa et al., 1999). Cytology is also essential in situations when culture is less likely to be positive because therapy has already been initiated. Microbiological culture and susceptibility results help direct therapy, but the practitioner should be cautious when interpreting susceptibility profiles: The "S" (susceptible), "I" (intermediate), and "R" (resistant) classification is based on achievable plasma antimicrobial concentrations. Since extremely high local drug concentrations are attained with topical or subconjunctival application, an antimicrobial may be effective despite the pathogen being classified as resistant by the diagnostic laboratory.

In cases of canine infectious conjunctivitis and keratitis, *Staphylococcus intermedius* or *Streptococcus* spp. are the most commonly identified pathogens, with Gram-negative bacteria identified less frequently (Tolar et al., 2006). This pattern of infection is likely to be similar in cats, with the addition of *Chlamydomydia felis* and *Mycoplasma felis*. In cases of equine infectious keratitis, there is initially an equal distribution of Gram-positive and Gram-negative bacteria (Moore et al., 1995a). The Gram-positive organisms are predominantly *Staphylococcus* spp. or *Streptococcus zooepidemicus*, but the Gram-negative bacteria most frequently isolated are *Pseudomonas* spp. Therefore, when treating horses, it is important to choose initial antimicrobial therapy that is effective against *Pseudomonas* spp. and other Gram-negative bacteria (Moore et al., 1995a; Keller and Hendrix, 2005). After initial antimicrobial treatment, isolation of Gram-negative bacteria increases and *Pseudomonas* spp. and *S. zooepidemicus* isolates are increasingly resistant to antimicrobials (Sauer et al., 2003).

In ruminants, there are several pathogens that may cause primary infectious keratitis, or "pinkeye". The major pathogens are *Moraxella bovis* in cattle, *Mycoplasma conjunctivae* and *Branhamella ovis* in goats and sheep, and *Chlamydomydia pecorum* in sheep.

Fungal infections of the eye are rare in dogs, cats and ruminants, but are frequently encountered in horses. Moore et al. (1995a) reported that 38% of equine infectious keratitis cases were caused by fungi. *Aspergillus* and *Fusarium* spp. are the most common fungi isolated from ulcerative keratomycosis in horses (Andrew et al., 1998).

Topical Drug Administration

Topically applied ophthalmic drugs distribute to the eye by three routes: transcorneal penetration, absorption by conjunctival blood vessels which flow into the ciliary body, and drainage and absorption through the nasolacrimal system. Transcorneal penetration is the most important consideration for therapy of ocular infections. Drainage and absorption through the nasolacrimal system contributes little to ocular therapy but is responsible for most adverse systemic effects. Commercial droppers deliver 25–50 μl /drop of solution or suspension, but only 10–25 μl are retained in the conjunctival fornix and tear film after immediate overflow. Therefore application of more than one drop at a time does not increase the drug concentration on the ocular surface. After 5 minutes, only 20% of the drug remains on the ocular surface; the rest is absorbed through the cornea and conjunctiva or removed by the nasolacrimal drainage system.

Because the epithelium-stroma-endothelium of the cornea is essentially a lipid-water-lipid sandwich, only drugs like chloramphenicol and fluoroquinolones that have both hydrophilic and lipophilic characteristics can penetrate the intact cornea easily. However, when trauma or a disease process disrupts normal corneal integrity, most antimicrobials achieve effective concentrations in the infected tissue.

Topical ophthalmologic drugs are formulated as ointments, solutions or suspensions. Deciding which formulation to use depends on several practical considerations. Ocular contact time of ointments is longer than solutions or suspensions, so they are more practical when the owner cannot follow a frequent administration regimen. Solutions and suspensions may be easier for some owners to apply than ointments. Avoid ointments on penetrating wounds or a descemetocoele, and prior to intraocular surgery, as their petroleum base elicits a severe granulomatous reaction when in direct contact with intraocular tissues.

The application frequency of topical antimicrobials depends on the disease and the drug formulation. One drop of an antimicrobial solution applied four times daily is usually sufficient for treatment of uncomplicated corneal ulcers and bacterial conjunctivitis. When ointments are used, a 5 mm strip is applied to the conjunctiva a minimum of three times a day. Severe ocular infections may need to be treated more frequently. Subpalpebral and nasolacrimal lavage systems are not well tolerated in small animals, but work well for in-

tensive topical therapy in horses. If more than one drug is involved in the therapeutic regimen, then 3 to 5 minutes should be allowed between application of each medication to avoid dilution or chemical incompatibility. Antimicrobial therapy is typically continued for seven days or until the ocular infection is resolved.

Topical Antimicrobials

There are few veterinary formulated ophthalmic antimicrobials available. There are a number of veterinary ophthalmic antimicrobial-corticosteroid combinations, but most ophthalmologists do not recommend the use of fixed-ratio antimicrobial-corticosteroid formulations. Corticosteroids are contraindicated with infectious keratitis, and ocular diseases that require corticosteroids to treat an inflammatory process typically do not require antimicrobial therapy. Many ophthalmology references recommend that practitioners compound drugs for ophthalmic use or “fortify” commercially available ophthalmic antimicrobials. Compounding drugs or adding injectable drugs to ophthalmic products carries the risks of chemical incompatibilities and contamination. This practice is usually unnecessary if an accurate diagnosis is made and an aggressive treatment regimen is instituted with commercially available ophthalmic products.

A first-choice antimicrobial for corneal ulcers and bacterial conjunctivitis or prophylaxis against surface infection is a “triple antibiotic”. Triple antibiotic ointment or solution contains neomycin, bacitracin and polymixin B. This combination provides broad-spectrum antimicrobial activity. These drugs are not lipid soluble, but penetrate the stroma when the corneal epithelium is disrupted. Neomycin is a typical bactericidal aminoglycoside with good activity against *Staphylococcus* spp. and Gram-negative bacteria. *Pseudomonas* spp. are often resistant to neomycin, but polymixin B is rapidly bactericidal against Gram-negative bacteria including *Pseudomonas* spp. Due to systemic toxicity, polymixin B is only used topically, so it is not typically included on susceptibility reports from microbiology services, but in a retrospective study, 100% of *Pseudomonas aeruginosa* isolates were susceptible to polymixin B (Hariharan et al., 1995). Polymixin B also binds and inactivates endotoxin, reducing inflammation and tissue destruction. The third component of triple antibiotic ointment is bacitracin. Like polymixin B, bacitracin is a topical product not

routinely included on susceptibility reports. Bacitracin is active against Gram-positive bacteria, with a mechanism of action similar to the beta-lactam antibiotics. Penicillins and cephalosporins are not used as commercial ophthalmic formulations due to the risk of contact sensitization, so bacitracin is their equivalent. Use of triple antibiotic was associated with selection for bacitracin-resistant *Streptococcus zooepidemicus* in cases of equine keratitis in one study (Keller and Hendrix, 2005).

Gentamicin is available as an ophthalmic solution and ointment. Because of its chemical characteristics, gentamicin does not readily cross lipid membranes, but it readily penetrates the stroma when the corneal epithelium is damaged. Gentamicin is a bactericidal aminoglycoside with activity against many Gram-negative pathogens, including many *Pseudomonas* spp. *Staphylococcus* spp. are usually susceptible to gentamicin (Moore et al., 1999b). *Pseudomonas* spp. and *Streptococcus zooepidemicus* may become resistant to gentamicin during therapy, so patients should be closely monitored for appropriate clinical response (Sauer et al., 2003). Microbiological culture and susceptibility testing should be repeated in nonresponsive cases.

Chloramphenicol is available in veterinary formulations as an ointment. Chloramphenicol is soluble in both water and lipid, so it penetrates the intact cornea with topical administration. Therefore, it is a good treatment choice for corneal stromal abscesses covered by intact epithelium. Chloramphenicol is a broad-spectrum, bacteriostatic antimicrobial, with excellent activity against *Chlamydomydia* and *Mycoplasma* spp. However, it is less effective than the aminoglycosides or fluoroquinolones against some Gram-negative bacteria and typically has poor efficacy against *Pseudomonas* spp. Chloramphenicol is a good first-choice antimicrobial for corneal ulcers and bacterial conjunctivitis in small animals. Because of the high incidence of *Pseudomonas* spp. in equine infectious keratitis, chloramphenicol is not an ideal choice for empirical therapy in horses.

Tetracycline ointment is a broad-spectrum, lipid soluble, bacteriostatic antimicrobial with good activity against the pathogens that cause infectious feline conjunctivitis and infectious keratoconjunctivitis in ruminants.

Erythromycin is available as a human-labelled ophthalmic ointment. As a macrolide, erythromycin is

lipid soluble and its spectrum of activity includes Gram-positive bacteria and *Mycoplasma* and *Chlamydomydia* spp. *Staphylococci* readily develop resistance to erythromycin. Erythromycin ointment is well-tolerated in cats.

Intramammary antimicrobial formulations are often used topically to treat infectious keratoconjunctivitis ("pink eye") in cattle.

Nonresponsive, progressive corneal ulceration results from infection with antimicrobial-resistant pathogens, including *Staphylococcus* spp. and *Pseudomonas* spp. Cytolytic toxins from staphylococci damage cell membranes and destroy polymorphonuclear leukocytes. *Pseudomonas* spp. exoproteins and enzymes released from neutrophils cause collagenolysis. Severe corneal infections from these pathogens may be treated with human-labelled formulations of tobramycin or a fluoroquinolone. Tobramycin is an aminoglycoside that is effective against most gentamicin-resistant *Pseudomonas* spp. and beta-lactamase-producing staphylococci. Ciprofloxacin and ofloxacin are human-labelled fluoroquinolone antimicrobials, with broad-spectrum bactericidal activity and high lipid solubility. They are effective against beta-lactamase-producing staphylococci and aminoglycoside-resistant *Pseudomonas* spp. Neither tobramycin nor the fluoroquinolones are very effective against streptococci. Because of their spectrum of activity, these antimicrobials should not be used for empirical treatment of ocular infections. Their use should be dictated by microbiological culture and susceptibility results.

Topical Antifungal Drugs

There are few antifungal drugs available for ophthalmic use, so fungal keratitis often requires compounding of other antifungal formulations. These cases are difficult to manage successfully and referral to a veterinary ophthalmologist is advised.

Miconazole is an imidazole derivative with broad antifungal activity. It is often considered the first choice for treatment of mycotic keratitis in horses because of its activity against *Aspergillus* spp. (Andrew et al., 1998). In the countries where there are available formulations, a 1% intravenous solution (10 mg/ml) is applied directly on the eye. Alternatively, the 2% veterinary dermatological cream may safely be applied directly to the eye. Miconazole lotions or sprays that contain ethyl alcohol should not be applied to the eye.

Under the direction of a veterinary ophthalmolo-

gist, other azole derivatives such as fluconazole, clotrimazole, and itraconazole can be formulated for the treatment of equine mycotic keratitis. An ointment formulated with 1% itraconazole and 30% dimethylsulfoxide has been successfully used in horses with mycotic keratitis (Ball et al., 1997). Amphotericin B may be used as a topical treatment of mycotic keratitis when there is resistance to other antifungal drugs, but this is a difficult drug to formulate properly for ophthalmic use. Natamycin is available in the United States as a 5% ophthalmic suspension. It has broad-spectrum activity against yeast and fungi and is the treatment of choice for *Fusarium* infections (Brooks et al., 1998).

Antiviral Ocular Drug Therapy

Herpes keratitis has only been well documented in the cat, but there are anecdotal reports in dogs and horses. Corticosteroids can accelerate the spread of viral infections, so they should not be administered concurrently. Clinically, some cats appear to respond to antiviral drugs (Andrew, 2001). However, herpes infections can go into remission without treatment, thus it is difficult to determine a specific antiviral treatment regimen that is clinically superior. All of the antiviral drugs are labelled for human use. The topical antivirals are static in action and topically irritating, so frequent administration is necessary and client compliance and patient tolerance are issues.

Trifluridine is incorporated in place of thymidine into viral DNA, resulting in faulty DNA and the inability to replicate or destroy tissue. Trifluridine penetrates the intact cornea, and ulceration and uveitis increase trifluridine's intraocular penetration. Trifluridine is administered 4–9 times daily for 2 days, and then the frequency is reduced over the next 2–3 weeks. If trifluridine is too irritating, then one of the other products may be tried.

Vidarabine ointment interferes with viral DNA synthesis. It is poorly lipid soluble, so corneal penetration is minimal unless ulceration is present. Suggested treatment is to apply a small amount of ointment five times daily until corneal re-epithelialization is complete, then every 12 hours for 7 days. Idoxuridine solution interferes with viral DNA replication by substituting for thymidine in the same manner as trifluridine. Idoxuridine does not penetrate the cornea unless the epithelial barrier is broken. Suggested treatment is to apply 1 drop every 4 hours until corneal re-

epithelialization occurs. Idoxuridine inhibits DNA formation in the cornea; therefore, prolonged or too frequent administration may damage the corneal epithelium and prevent ulcer healing.

Subconjunctival Antimicrobial Therapy

Drug injection into the bulbar subconjunctival space avoids tear dilution and directly bypasses the conjunctival epithelial barrier, rapidly delivering a high concentration in the anterior segment of the eye. Medications injected into the subconjunctival space reach the anterior segment directly through the ciliary circulation and indirectly by leaking from the injection site to be absorbed through the cornea and the conjunctiva. Antimicrobials should not be placed under the palpebral conjunctiva, as blood circulation in this area is directed away from the eye. Therapeutic antimicrobial concentrations are usually maintained for 3 to 6 hours after a subconjunctival injection, then decrease slowly over the next 24 hours.

Subconjunctival injections are indicated if frequent topical application cannot be done. Severe conjunctival irritation may occur with repeated daily injections. Other potential complications include granuloma formation and inadvertent intraocular or intra-scleral injection. The antimicrobials most often used for subconjunctival injection are penicillins, cephalosporins, gentamicin, and miconazole.

Systemic Antimicrobial Therapy

Systemic administration is necessary to achieve therapeutic drug concentrations in the lids, lacrimal system, orbit, and posterior ocular segment. The passage of drugs into the eye is normally limited by the blood-ocular barrier. Concentrations attained in the aqueous humour are often similar to those attained in the cerebrospinal fluid, reflecting similarities between the blood-ocular and blood-brain barriers. As in the blood-brain barrier, inflammation disrupts the blood-ocular barrier and improves drug penetration. Because high peak plasma concentrations promote the passage of antimicrobials into the eye, intravenous administration is preferable to oral, intramuscular or subcutaneous routes.

Initial antimicrobial therapy should be chosen on the basis of cytologic evaluation of fine needle aspirates from the infected eye, eyelid, or orbit. Choice of therapy should be re-evaluated when culture and susceptibility information is available. Bacterial endoph-

thelmitis associated with surgical contamination is often due to Gram-positive bacteria, thus cefazolin is appropriate for surgical prophylaxis. Traumatic perforations of the eye may involve both Gram-positive and Gram-negative bacteria, so a beta-lactam combined with a fluoroquinolone would be a rational choice. Bacterial blepharitis, dacryocystitis and orbital cellulitis are likely due to skin flora such as *Staphylococcus* spp., so beta-lactamase-resistant antimicrobials such as cephalexin or amoxicillin/clavulanic acid are appropriate first choices for therapy. Systemic administration of oxytetracycline produces adequate concentrations in tears for the effective treatment of the organisms that cause infectious keratoconjunctivitis in ruminants (Brown, et al. 1998).

Bibliography

- Andrew SE, et al. 1998. Equine ulcerative keratomycosis: visual outcome and ocular survival in 39 cases (1987–1996). *Equine Vet J* 30:109.
- Andrew SE. 2001. Ocular manifestations of feline herpesvirus. *J Feline Med Surg* 3:9.
- Ball MA, et al. 1997. Evaluation of itraconazole-dimethyl sulfoxide ointment for treatment of keratomycosis in nine horses. *J Am Vet Med Assoc* 211:199.
- Brooks DE, et al. 1998. Antimicrobial susceptibility patterns of fungi isolated from horses with ulcerative keratomycosis. *Am J Vet Res* 59:138.
- Brown MH, et al. 1998. Infectious bovine keratoconjunctivitis: a review. *J Vet Intern Med* 12:259.
- Hariharan H, et al. 1995. Antimicrobial drug susceptibility of clinical isolates of *Pseudomonas aeruginosa*. *Can Vet J* 36:166.
- Keller RL, Hendrix DV. 2005. Bacterial isolates and antimicrobial susceptibilities in equine bacterial ulcerative keratitis (1993–2004). *Equine Vet J* 37:207.
- Massa KL, et al. 1999. Usefulness of aerobic microbial culture and cytologic evaluation of corneal specimens in the diagnosis of infectious ulcerative keratitis in animals. *J Am Vet Med Assoc* 215:1671.
- Moore CP, et al. 1995a. Antibacterial susceptibility patterns for microbial isolates associated with infectious keratitis in horses: 63 cases (1986–1994). *J Am Vet Med Assoc* 207:928.
- Moore CP, et al. 1995b. Antimicrobial agents for treatment of infectious keratitis in horses. *J Am Vet Med Assoc* 207:855.
- Sauer P, et al. 2003. Changes in antibiotic resistance in equine bacterial ulcerative keratitis (1991–2000): 65 horses. *Vet Ophthalmol* 6:309.
- Tolar EL, et al. 2006. Evaluation of clinical characteristics and bacterial isolates in dogs with bacterial keratitis: 97 cases (1993–2003). *J Am Vet Med Assoc* 228:80.

Bacterial Meningitis

Patricia M. Dowling

Sixty years after the introduction of antimicrobials, bacterial meningitis remains an important cause of mortality and morbidity in human beings and animals (Fecteau and George, 2004; Meric, 1988; Moore, 1995; Yogeve and Guzman-Cottrill, 2005). Bacterial meningitis is unique among infectious diseases in that clinical outcome is suboptimal despite bacteriologic cure of the infection. Despite advances in the diagnosis and treatment of neonatal bacterial septicemia, mortality rates of 100% for bacterial meningitis are reported in animals (Green and Smith, 1992). Powerful, broad-spectrum antimicrobials have not improved the outcome of bacterial meningitis because the inflammatory host response to bacterial products continues after the bacteria are killed with antimicrobials. The host response damages tissues and contributes significantly to central nervous system (CNS) injury. Treatment of bacterial meningitis in human medicine now utilizes “partner drugs” to both kill bacteria and limit the detrimental effects of the immune response in the CNS (van der Flier et al., 2003).

The optimal approach to treating bacterial meningitis in animals consists of early detection of clinical signs, rapid determination of the pathogen(s) involved and their antimicrobial susceptibility, selection of an antimicrobial that achieves therapeutic concentrations in the cerebrospinal fluid (CSF), and administration of drugs to moderate the potentially destructive immune response. Underlying deficiencies such as failure of passive transfer of antibodies also need to be corrected.

Pathogenesis of Bacterial Meningitis

Meningitis is a complex infection that differs pathophysiologically from peripheral bacterial infections. In large animals, meningitis usually occurs in neonates secondary to septicemia and bacteremia associated with failure of transfer of passive immunity. As meningitis is a localized manifestation of septicemia, concurrent problems commonly include omphalophlebitis, panophthalmitis, polyarthritis, pneumonia, and enteritis.

In order to cause meningitis, bacterial pathogens must sequentially invade and survive in the intravascular space, cross the blood-brain barrier (BBB), and survive in the CSF (Webb and Muir, 2000). The initial

host defense against sustained bacteremia is circulating complement, particularly through the alternative complement pathway that does not require specific antibody for activation. Evasion of the alternative complement pathway allows bacteria to survive in circulation. After successful hematogenous dissemination, bacteria are transported to the CNS and localize in the choroid plexus. Subsequently, bacteria enter the ventricular system and are transferred to the subarachnoid space via normal CSF flow.

The least understood step in the pathogenesis of meningitis is the mechanism of bacterial penetration of the BBB and entry into the CSF (Tuomanen, 1993). Work with bioactive cell wall fragments from pneumococcus suggests a single glycopeptide to be responsible for allowing bacterial penetration of the BBB. For *E. coli*, an important cause of foal and calf meningitis, fimbriation appears to be an important virulence factor.

Once bacteria gain access to the CSF, defenses against bacterial invasion are limited, allowing rapid and unchecked bacterial proliferation. Humoral defenses, particularly immunoglobulins and complement, are virtually absent from the CSF. These components of inflammatory defense must be derived from serum. Opsonic activity is undetectable in normal CSF and increases inconsistently during BBB breakdown. The inflammatory response appears in the CSF only when a threshold amount of bacterial components (approximately 10^5 bacteria) is reached.

Brain injury and neuronal death due to bacterial meningitis involve a combination of altered cerebral metabolism, cerebral edema, increased intracranial pressure, decreased cerebral blood flow, altered CSF dynamics, and leukocyte-mediated injury to neuronal tissue (van der Flier et al., 2003). Gram-negative lipopolysaccharide (LPS), Gram-positive peptidoglycan, and cytotoxins engage Toll-like receptors of the endothelia and activate their downstream signaling cascades. The endothelial cells then release mediators, including tumor necrosis factor alpha, nitric oxide, and matrix metalloproteinase-2, which increase endothelial permeability. The endothelium expresses multiple leukocyte adhesion molecules and presents chemotactic factors such as interleukin-8 (IL-8) when activated by inflammatory mediators. This combination promotes neutrophil adherence and transendothelial migration. Up-regulation of endothelial tissue factor triggers a procoagulant state and stimulates

thrombus formation. Endothelial activation and release of vasoconstrictors, such as the endothelins, and vasodilators, such as nitric oxide, impair autoregulation of cerebral perfusion pressure. Along with systemic hypotension in critically ill patients, these events further decrease cerebral perfusion and exacerbate neuronal death.

Etiology of Meningitis

The etiology and epidemiology of bacterial meningitis vary with species. Bacterial meningitis in adult dogs and cats usually arises from hematogenous spread of distal infections (enteritis, prostatitis, metritis, pneumonia) or from direct extension of non-CNS infections such as otitis interna (Cook et al., 2003; Radaelli and Platt, 2002; Spangler and Dewey, 2000). A wide range of bacteria have been isolated from feline and canine meningitis cases, including *E. coli*, *Klebsiella* spp., *Staphylococcus* spp., *Streptococcus* spp., *Pasteurella* spp., *Actinomyces* spp., *Nocardia* spp., and various anaerobic species including *Peptostreptococcus*, *Eubacterium*, *Fusobacterium*, and *Bacteroides* spp. Ehrlichial and rickettsial organisms can also cause meningitis in small animals.

Bacterial meningitis commonly occurs in neonatal large animals as a sequela to failure of transfer of passive immunity. Meningitis is predominantly caused by Gram-negative enteric pathogens (*E. coli* and *Salmonella* spp.) and beta-hemolytic streptococci in septic foals, and *Streptococcus* spp. in older horses (Moore, 1995). Bacterial meningitis has been reported from direct extension of cranial infections and in association with common variable immunodeficiency in adult horses (Pellegrini-Masini et al., 2005; Smith et al., 2004).

The Enterobacteriaceae are the cause of most cases of bacterial meningitis in septic ruminant neonates. Along with polyarthritis and pneumonia, *Mycoplasma bovis* can cause meningitis in young calves (Stipkovits et al., 1993). In adult cattle and sheep, meningoencephalomyelitis is caused by *Histophilus somni*, and encephalitis by *Listeria monocytogenes* (Braun et al., 2002; Fecteau and George, 2004). Pituitary abscesses in cattle are caused by *Arcanobacterium pyogenes* and anaerobic bacteria.

While enteric Gram-negative pathogens cause meningitis in septic piglets, the most common cause of infectious meningitis in pigs is *Streptococcus suis* type 2 (Gottschalk and Segura, 2000).

Therapy of Bacterial Meningitis

Infections of the CNS are associated with high morbidity and mortality. Treatment failure in septic neonates is attributed to the advanced state of disease when diagnosed, the limited ability of antimicrobials to cross the blood-brain barrier, and development of antimicrobial-resistant bacteria. The CSF penetration of an antimicrobial depends on the integrity of the BBB and the physical and chemical characteristics of the drug. In order to achieve therapeutic concentrations, antimicrobials for therapy of CNS infections should be lipid soluble, of low molecular weight, have a low degree of protein binding, and be weak bases to take advantage of ion trapping.

For example, beta-lactam antibiotics poorly penetrate the normal BBB, achieving CSF concentrations only 0.5–2.0% of peak serum concentrations. These weak organic acids are also actively transported out of the CNS against a concentration gradient. However, this mechanism is disrupted by meningeal inflammation. Inflammation also increases separation of intercellular tight junctions and vesicular transport, so that penetration of the BBB is significantly enhanced (up to 55% of peak serum concentrations).

The poor host defense mechanisms in the CNS suggest that only antimicrobials that achieve bactericidal concentrations in the CSF should be used for therapy of meningitis. However, highly bactericidal antimicrobials do not necessarily improve clinical outcome. The bacterial cell wall of Gram-positive bacteria and endotoxin released from Gram-negative bacteria stimulate a dramatic inflammatory response.

Reports from human medicine indicate that improved clinical outcome does not come from “better” bactericidal drugs, but from treatments targeting the pathogenesis of CSF inflammation. Newer beta-lactams such as imipenem lyse bacteria in a manner that does not create the same high concentrations of inflammatory debris seen with conventional beta-lactam antibiotics (Tuomanen, 1993).

“Partner drugs” can be administered to decrease the detrimental inflammatory response. Work is being done with antibodies that capture the inflammatory cell wall pieces in order to render them inert. To decrease leukocyte damage during inflammation, antibodies that block leukocyte adhesion to endothelia and prevent accumulation of leukocytes in cerebrospinal fluid are being investigated. Steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs)

down-modulate cerebrospinal fluid leukocytosis, chemical abnormalities, and pressure changes, and reduce cerebral edema. The precise dosing and timing of these “partner drugs” are critical. For example, corticosteroids are beneficial in some types of meningitis in children when administered early, but have no effect or are detrimental when administered later (King SM, et al. 1994).

Little is known about the best use of “partner drugs” in animals, but failure of antibody transfer can be corrected in large animal neonates with plasma transfusions. The risks and benefits of administering corticosteroids or NSAIDs need further investigation in veterinary patients with meningitis.

Antimicrobial choice should be based on CNS penetration and the initial results of Gram stain followed by culture and susceptibility results from CSF or other infected tissues. Antimicrobials should be administered intravenously to attain maximum peak plasma concentrations and thus provide a concentration gradient to aid passage of drugs into the CNS. For therapy with beta-lactams, aminoglycosides, and fluoroquinolones, there is a significant correlation between increasing the drug concentration in the CSF and increasing bacterial killing rates (Yogev and Guzman-Cottrill, 2005). For these antimicrobials, maximal bactericidal activity occurs when the CSF concentration is 10–30 times higher than the *in vitro* minimal bactericidal concentration (MBC). The maximum bactericidal activity of vancomycin occurs when CSF concentrations are 5–10 times higher than the MBC. In contrast, increasing the CSF concentration of rifampin does not increase killing rate. To ensure optimal penetration of antimicrobials into the CNS, intravenous dosing should be maintained for the entire treatment course. As meningeal inflammation decreases with therapy, penetration of some drugs across the BBB diminishes (e.g., CSF penicillin concentrations are reduced by almost 50% on day 5 of therapy compared with day 1) (Yogev and Guzman-Cottrill, 2005). Apparently effective therapy should be continued for 7 to 14 days, but shorter times may be sufficient with antimicrobials that rapidly sterilize the CSF, such as the newer cephalosporins.

Beta-lactam Antibiotics

Penicillins and first generation cephalosporins may be effective for bacterial meningitis from sensitive Gram-positive bacteria, such as *Streptococcus* spp., *Listeria*

monocytogenes and anaerobes. Because it is highly protein bound, ceftiofur does not reach therapeutic concentrations in the CSF. As enteric bacteria are often resistant to ampicillin or amoxicillin, these aminopenicillins are not recommended in the treatment of meningitis in large animal neonates. Because of their high bactericidal activity against Enterobacteriaceae, third generation cephalosporins are preferred for treatment of meningitis in septic neonates. Moreover, their use in large animals is limited to neonates due to the expense of therapy. Third generation cephalosporins are more active against Gram-negative bacteria than earlier cephalosporins, but no more active against Gram-positive bacteria. Cefotaxime, ceftazidime, ceftizoxime and ceftriaxone consistently reach effective antibacterial concentrations in the CNS in humans with inflamed meninges.

Potentiated Sulfonamides

Sulfonamides are commonly administered in conjunction with a diaminopyrimidine to take advantage of synergistic antimicrobial activity and to reduce the development of antimicrobial resistance. These "potentiated" sulfonamides have broad-spectrum activity against *Streptococcus* spp., *E. coli*, *Proteus*, *Pasteurella*, *Histophilus*, and *Salmonella* spp. Staphylococci, anaerobes, *Nocardia*, *Corynebacterium*, *Klebsiella*, and *Enterobacter* spp. are initially susceptible but may become resistant. Because of frequent use, resistance to trimethoprim-sulfonamide combinations frequently occurs in bacteria isolated from septicemic foals and calves, so its use is not recommended without confirmation from susceptibility test results. Trimethoprim-sulfonamide combinations are effective for treatment of *S. suis* type 2 meningitis in pigs. Sulfonamides are well distributed throughout the body, and a few penetrate into the CSF, depending on degree of protein binding and pKa values. Ormetoprim-sulfadimethoxine, trimethoprim-sulfadiazine, and trimethoprim-sulfamethoxazole are all well distributed into the CSF. Meningeal inflammation does not alter this distribution. With chronic dosing, sulfamethoxazole accumulates in the CSF, but trimethoprim does not.

Tetracyclines

Tetracyclines are lipid soluble and well distributed to most tissues, but do not readily reach therapeutic concentrations in the CSF for most causes of bacterial

meningitis. Doxycycline is the most lipid soluble tetracycline and has the greatest degree of CSF penetration. High intravenous doses of oxytetracycline may be effective for early treatment of meningitis due to *Listeria monocytogenes* in ruminants, but resistance has been documented (Vela et al., 2001).

Chloramphenicol and Florfenicol

Chloramphenicol is a bacteriostatic, broad-spectrum antimicrobial with activity against many Gram-positive, Gram-negative and anaerobic bacteria. Its bacteriostatic action may contribute to its efficacy, as it does not cause an explosive release of endotoxin or cell wall fragments. Due to lipid solubility and low protein binding, chloramphenicol is widely distributed throughout the body and achieves CSF concentrations up to 50% of plasma concentrations when the meninges are normal and more if inflammation is present. Because of human health concerns, chloramphenicol has been replaced for many diseases in veterinary medicine by the fluoroquinolones, and availability of chloramphenicol products is limited. If given intravenously, florfenicol penetrates well into CSF, with concentrations in the CSF reaching 46% of plasma concentrations. The CSF concentrations remain above the MIC for *Histophilus somni* for over 20 hours, but concentrations above the MIC values for Gram-negative enteric pathogens are not achieved (de Craene et al., 1997).

Fluoroquinolones

Fluoroquinolones penetrate well into the CSF during meningitis, reaching CSF concentrations 20–50% of plasma concentrations. They are potentially useful for meningitis in patients with resistant Gram-negative bacteria that do not respond to beta-lactam drugs. Enrofloxacin is available in injectable formulations for IM use in small animals and SC use in cattle, but these formulations may also be administered by slow IV injection. Enrofloxacin may be less expensive for therapy of meningitis in large animals than third generation cephalosporins. Ciprofloxacin is available in human IV formulations, but may be cost prohibitive for use in large animals. In the United States, the extra-label use of fluoroquinolones in food animals is strictly prohibited. Enrofloxacin is highly lipid soluble, and may attain therapeutic concentrations in the CSF for Gram-negative pathogens such as *E. coli*, *Salmonella* spp., *Actinobacillus* spp. and *Klebsiella* spp. Fluoroquino-

lones have variable efficacy against streptococci and no activity against anaerobic bacteria. Use of enrofloxacin in neonatal foals has been documented to cause arthropathies, but because therapy with enrofloxacin is economical and effective, it may still be the treatment of choice in life-threatening cases of sepsis and meningitis.

Macrolides and Lincosamides

The macrolides and lincosamides are typically active against Gram-positive bacteria, some Gram-negative respiratory tract pathogens, and anaerobes. They are not active against the Enterobacteriaceae. Erythromycin, clarithromycin, azithromycin and clindamycin concentrate in leukocytes, making them very effective against intracellular pathogens. Lincomycin and clindamycin penetrate into the CNS better than the macrolides. Erythromycin has been used in children with penicillin-resistant *Streptococcus pneumoniae* meningitis, but resistance is common. Erythromycin and clindamycin are available as human IV formulations. Early treatment of bovine respiratory disease with tilmicosin or tulathromycin may prevent thromboembolic meningoencephalitis from *Histophilus somni* in cattle. Advanced cases with microabscesses and thrombophlebitis in the CNS are unlikely to respond.

Rifampin

Rifampin is a highly lipid soluble antimicrobial with activity against Gram-positive and anaerobic bacteria, including streptococci, *Rhodococcus equi*, *Staphylococcus aureus*, and *Mycobacterium* spp. Because bacterial resistance rapidly emerges to rifampin, it is commonly used in conjunction with other antimicrobials. Rifampin widely distributes in tissues and the CSF. It is most commonly used as the oral formulation in combination with a macrolide to treat *Rhodococcus equi* infections in foals. However, there are human intravenous formulations that could be used, providing the dose is corrected for bioavailability.

Metronidazole

Metronidazole is highly effective against anaerobic bacteria, including *Bacteroides fragilis* (penicillin-resistant strains), *Fusobacterium*, and *Clostridium* spp. Metronidazole is very lipid soluble and readily penetrates into the brain and CSF. It is available in human intravenous formulations, but these formulations may

be cost prohibitive for large animals. In the United States and Canada, metronidazole is strictly prohibited for use in food animals.

Bibliography

- Braun U, et al. 2002. Clinical findings and treatment of listeriosis in 67 sheep and goats. *Vet Rec* 150:38.
- Cook LB, et al. 2003. Inflammatory polyp in the middle ear with secondary suppurative meningoencephalitis in a cat. *Vet Radiol Ultrasound* 44:648.
- De Craene BA, et al. 1997. Pharmacokinetics of florfenicol in cerebrospinal fluid and plasma of calves. *Antimicrob Agents Chemother* 41:1991.
- Fecteau G, George LW. 2004. Bacterial meningitis and encephalitis in ruminants. *Vet Clin North Am Food Anim Pract* 20:363.
- Gottschalk M, Segura M. 2000. The pathogenesis of the meningitis caused by *Streptococcus suis*: the unresolved questions. *Vet Microbiol* 76:259.
- Green SL, Smith LL. 1992. Meningitis in neonatal calves (1983–1990). *J Am Vet Med Assoc* 201:125.
- King SM, et al. 1994. Dexamethasone therapy for bacterial meningitis: better never than late? *Can J Infect Dis* 5:210.
- Meric SM. 1988. Canine meningitis: a changing emphasis. *J Vet Intern Med* 2:26.
- Moore BR. 1995. Bacterial meningitis in foals. *Comp Cont Educ Pract Vet* 17:1417.
- Pellegrini-Masini A, et al. 2005. Common variable immunodeficiency in three horses with presumptive bacterial meningitis. *J Am Vet Med Assoc* 227:114.
- Radaelli ST, Platt SR. 2002. Bacterial meningoencephalomyelitis in dogs: a retrospective study of 23 cases (1990–1999). *J Vet Intern Med* 16(2):159.
- Spangler EA, Dewey CW. 2000. Meningoencephalitis secondary to bacterial otitis media/interna in a dog. *J Am Anim Hosp Assoc* 36:239.
- Smith JJ, et al. 2004. Bacterial meningitis and brain abscesses secondary to infectious disease processes involving the head in horses: seven cases (1980–2001). *J Am Vet Med Assoc* 224:739.
- Stipkovits L, et al. 1993. Mycoplasmal arthritis and meningitis in calves. *Acta Vet Hung* 41:73.
- Tuomanen E. 1993. Breaching the blood-brain barrier. *Sci Am* 268:80.
- van der Flier M, et al. 2003. Reprogramming the host response in bacterial meningitis: how best to improve outcome? *Clin Microbiol Rev* 16:415.
- Vela AI, et al. 2001. Antimicrobial susceptibility of *Listeria monocytogenes* isolated from meningoencephalitis in sheep. *Int J Antimicrob Agents* 17:215.
- Webb AA, Muir GD. 2000. The blood-brain barrier and its role in inflammation. *J Vet Intern Med* 14:399.
- Yogev R, Guzman-Cottrill J. 2005. Bacterial meningitis in children: critical review of current concepts. *Drugs* 65:1097.

Urinary Tract Infections

Stephen Kruth

Introduction

Bacterial urinary tract infections are the most common cause of urinary tract disease in dogs. Approximately 14% of all dogs will acquire a bacterial urinary tract infection (UTI) during their lifetimes and approximately 10% of dogs presented to the veterinarian for other problems will have a concurrent bacterial UTI (Ling, 1993).

Studies of feline lower urinary tract disease suggest that the prevalence of bacterial UTI is less than 5% in cats presenting with an initial episode of signs related to urinary tract disease (Buffington et al., 1997; Hostutler et al., 2005). One retrospective study evaluating the prevalence of bacterial UTI in cats referred to a teaching hospital found that 25% of feline urine samples were infected. These findings suggest that the prevalence of bacterial UTI in cats may not be as low as previously reported. The mean age of cats in the study was 8.2 years and it was hypothesized that older cats may be more susceptible to bacterial UTI because of diminished host defenses secondary to aging or concomitant disease (diabetes mellitus, renal failure, hyperthyroidism).

Persistent or relapsing infections occur when the original infection is not fully resolved despite therapy. Reinfection occurs when a new bacterial species or strain infects a patient after a period of urine sterility. Superinfections occur when a different organism colonizes the urinary tract during the course of treatment for the original infection. The clinical and microbiological characteristics of these infections in dogs have been described (Norris, 2000; Sequin, 2003; Drazenovich et al., 2004).

The consequences of bacterial UTI in dogs and cats can be significant if the infection goes undiagnosed and untreated. Because many cats and dogs with UTI do not display clinical signs or do not have detectable bacteriuria or pyuria, diagnosis may be incidental in some cases. Colonization of any part of the urinary tract with bacteria makes the animal susceptible to infection in other parts of the urinary tract and body. Potential consequences of undiagnosed UTI include infertility, urinary incontinence, discospondylitis, pyelonephritis, and renal failure. Septicemia can occur as a consequence of UTI in immunosuppressed pa-

tients. In intact male dogs, bacterial UTI often extends to the prostate, and occasionally to the spermatic cords or testicles. Bacterial prostatitis can cause infertility, abscess formation and recurrent UTI. In dogs, infection of the urine with urea-splitting bacteria (*Staphylococcus intermedius* and *Proteus mirabilis*) is often associated with the formation of struvite uroliths. *Corynebacterium urealyticum*, also a rapid urea-splitting organism, is associated with alkaline urine and struvite and calcium phosphate precipitation, which can result in bladder wall encrustations (Bailliff et al., 2005).

Pathogenesis

The establishment of bacterial UTI in dogs and cats is primarily dependent upon the interaction between host defenses and bacterial virulence factors. Studies in cats and dogs have shown that when host defenses are altered by catheterization, surgery, or other diseases of the urinary tract (idiopathic cystitis, urolithiasis, polyps, neoplasia, etc.), the incidence of bacterial UTI is high (Barsanti et al., 1992; Martinez, 2003). Abnormalities of host defenses are thought to be the most important factors in the pathogenesis of UTI and the persistence of complicated UTI. Defense mechanisms of the host can be found in all areas of the urinary tract including the urethra, bladder, ureters, kidneys and urine itself.

The most common route of infection in dogs and cats is through ascent of microorganisms within the urethra. Normal bacterial flora of the distal urethra are thought to compete with invading uropathogenic bacteria through bacterial interference. In the normal urethra, the resident flora may consume essential nutrients, interfere with bacterial adhesion to the uroepithelium or secrete bacteriocins, thus preventing the uropathogen from colonizing the urethra. In addition, the surface of the urethra has intrinsic properties that prevent bacterial colonization. Scanning electron microscopy of the urethra in female dogs reveals that the uroepithelium of the distal urethra and vagina has surface microvilli that allow for the attachment of resident bacteria. In contrast, the surface of the proximal urethra and bladder has microplicae, folds present when the lumen of the urethra is contracted. These folds flatten when the lumen of the urethra is distended during the act of micturition, thus making it difficult for bacteria to adhere. Such structural differences between epithelia may be associated with resist-

ance to bacterial colonization. Another host defense of the urethra involves the production of secretory IgA, which prevents bacterial adherence and colonization. Intrinsic properties of the urethra such as urethral peristalsis and a functional high-pressure zone in the mid-urethra also act to prevent bacterial colonization.

Micturition is an important defense against bacterial colonization of the lower urinary tract. Frequent voiding of adequate amounts of urine removes ascending bacteria that gain access to the urethra. In addition, the flattening of urethral folds may dislodge adherent bacteria during voiding. The production of fresh urine dilutes bacterial counts in urine and complete voiding helps empty the bladder of bacteria. Some diseases of the urinary tract such as bladder atony, urolithiasis, and prolonged urine retention predispose to infection because of the presence of residual urine.

The antibacterial properties of urine have been studied extensively in humans, dogs, rabbits and cats. Although urine does support bacterial growth, there are components of the urine that are antibacterial, including a very acidic or alkaline pH, a high concentration of urea, a high osmolality, and some weak organic acids. Urine oligosaccharides and Tamm-Horsfall protein entrap bacteria and prevent bacterial colonization. Changes in the urine composition predispose some patients to urinary tract infection. For example, diuresis decreases the osmolality of urine and may decrease the antibacterial properties of urine. Urine dilution as well as an impaired immune response may contribute to the development of UTI in dogs receiving corticosteroids (Ihrke et al., 1985; Torres et al., 2005). In addition, excessive amounts of glucose in the urine inhibit phagocytosis and may predispose to bacterial colonization of the urothelium. Dogs with diabetes mellitus or hyperadrenocorticism are at increased risk for urinary tract infection, and may have asymptomatic bacteriuria. Dogs with these disorders should have their urine cultured even if clinical signs and urinalysis findings are not suggestive of UTI (McGuire et al., 2002; Chastain et al., 2003).

Anatomical abnormalities of the lower urinary tract such as vulvar abnormalities and urethrostomies, as well as indwelling catheters and cystotomy tubes, are risk factors for ascending bacterial infections (Stiffler et al., 2003; Smarick et al., 2004).

The anatomy and function of the ureters provide a mechanism of defense against bacterial invasion of the

kidneys. The distal ureter courses through the bladder wall at an angle that forms a one-way flap, thus preventing vesicoureteral reflux. Peristalsis of the ureters promotes unidirectional flow of urine from the kidneys to the bladder and is an important defense against the migration of bacteria that are able to ascend the ureters independent of vesicoureteral reflux.

Renal defenses are primarily local and systemic immune responses. The renal cortex is much less susceptible to infection than is the medulla, possibly due to higher blood flow in the cortex. Several factors, including lower blood flow, high ammonia concentrations, and increased osmolality, interfere with local immune responses in the medulla, thus making it more susceptible to infection.

In addition to a disruption of the normal host defenses, certain bacterial virulence factors enhance colonization of the urinary epithelium and allow the development of UTI. Bacterial adhesion to the uroepithelium is thought to be the most important virulence factor of uropathogenic organisms. *E. coli* and *Proteus mirabilis* both have specific fimbriae that enhance bacterial adherence to the epithelial surface of the urinary tract (Senior et al., 1992; Gaastra et al., 1996). Many of the common uropathogens, including *E. coli*, *Proteus* spp., *Staphylococcus* spp., and *Pseudomonas* spp., carry R-plasmids which confer bacterial resistance to antimicrobial agents, enhancing their pathogenicity. Other virulence factors include capsules that surround bacteria and limit phagocytosis, antibody coating, and opsonization. In addition, *E. coli* produces factors such as hemolysin and aerobactin that promote bacterial growth (Wilson, 1988). Analysis of *E. coli* virulence factors suggests that some canine isolates cause both cystitis and diarrhea, and that these strains may be infectious for humans (Johnson et al., 2001; Johnson, 2003; Starcic, 2002).

Route of Infection

The most common route of infection in dogs and cats is the ascending migration of bacteria through the urethra. In humans and dogs, the normal flora of the genital, rectal and perineal areas has been shown to be the primary reservoir of infection (Low, 1988). The normal flora of the prepuce and vagina has been well described. The most frequently isolated bacterium from dogs with UTI is *E. coli*, followed by *Staphylococcus* spp., *Proteus* spp., *Streptococcus* spp., *Klebsiella* spp., and *Pseudomonas* spp. (Table 22.1) (Ling, 2001;

Table 22.1. Bacteria isolated from urinary tract infections in dogs and cats, with suggested antimicrobial therapies.

Pathogen	Dogs (%)	Cats (%)	Suggested antimicrobials
<i>Escherichia coli</i>	43	47	Amoxicillin-clavulanate, trimethoprim-sulfa, cephalixin
<i>Staphylococcus</i> spp.	20	18	Amoxicillin-clavulanate, ampicillin, cephalixin
<i>Streptococcus</i> spp.	11	13	Ampicillin, amoxicillin-clavulanate
<i>Proteus</i> spp.	13	4	Ampicillin, amoxicillin-clavulanate
<i>Klebsiella</i> spp.	4	4	Cephalexin, trimethoprim-sulfa, amoxicillin-clavulanate
<i>Enterobacter</i> spp.	3	1	Trimethoprim-sulfa, amoxicillin-clavulanate
<i>Pseudomonas aeruginosa</i>	2	1	Fluoroquinolones, tetracycline
<i>Pasteurella</i> spp.	—	2	Ampicillin
<i>Mycoplasma</i> spp.	—	1	Tetracycline, fluoroquinolones

Prescott et al., 2002). A significant increase in the proportion of enterococcal UTIs since 1984 has been reported (Prescott et al., 2002). *Mycoplasma* spp. were isolated as a single agent in 68% of dogs presented for signs of UTI in one study, suggesting that *Mycoplasma* spp. may be an important uropathogen in some cases (Jang, 1984). In cats, *E. coli* is the most frequently isolated organism and is found in up to 50% of infected urine samples. *Staphylococcus* spp. and *Streptococcus* spp. are the second most frequently isolated, followed infrequently by other opportunist pathogens.

Clinical Manifestations

Clinical signs associated with bacterial urinary tract infections in dogs and cats are variable and are dependent upon the site and duration of infection, the number and virulence of the bacteria, and the response of the host to the invading organism. In animals that are immunosuppressed or have chronic disease, bacterial UTI may be asymptomatic. History and physical examination findings assist in localizing disease to the urinary tract, but are not specific for bacterial UTI. Historical findings associated with UTI of the lower urinary tract include dysuria, pollakiuria, incontinence, hematuria, and abnormal urine odor or color. As a singular problem, infection confined to the lower urinary tract will not cause systemic signs of disease. Historical findings associated with infection of the upper urinary tract usually include polyuria and polydipsia due to bacterial toxins that act in the distal tubule to inhibit ADH activity. Signs of systemic disease may or may not be present. Physical examination of the dog or cat with an upper or lower urinary tract infection may reveal no abnormal findings. Infection of the lower urinary tract may be associated with a

small, firm, thickened and painful bladder on palpation. Infection of the upper urinary tract may cause fever, abdominal pain (localized to the kidney), and palpably enlarged or normal sized kidneys.

Diagnosis

Urinalysis

Infection of the urinary tract is confirmed by examination and culture of bladder urine. Knowledge of the method of urine collection and sample handling are crucial in diagnostic evaluation of urine. In a study comparing various methods of urine collection, all specimens from normal dogs collected by antepubic cystocentesis were bacteriologically sterile. Urine samples from normal dogs collected by catheterization and voided specimens had 26% and 85% bacterial growth, respectively (Comer and Ling, 1981). Patients who are immunosuppressed may be at greater risk for iatrogenic UTI if urinary catheterization is performed. White blood cells and protein, as well as bacteria that are normally present at the external urethral orifice, can contaminate specimens collected by means other than cystocentesis, making interpretation of bacteriuria, pyuria, and proteinuria in these samples difficult. Cystocentesis is a well-described method of urine collection, has a low incidence of complications, and is the only method that yields a specimen free of contaminants.

Samples collected for urinalysis should be processed within 15 minutes or refrigerated immediately. Specimens for quantitative culture can be refrigerated for up to 6 hours without significant increases in bacterial numbers (Padilla, 1981). Refrigeration and prolonged storage can alter the morphology of crystals in the urine, alter urine pH, and kill some fastidious uropathogens.

Urine sediment should be evaluated for the presence of white blood cells, red blood cells, and bacteria, findings consistent with inflammation and possibly infection. Examination of modified Wright-stained preparations of urine sediment has significantly improved sensitivity, specificity, positive predictive value, and test efficiency of light-microscopic detection of bacteriuria, compared with the routine unstained method (Swenson, 2004). Samples collected by cystocentesis that have evidence of inflammation localize the disease to the bladder, ureters, or kidneys. Normal urine collected by cystocentesis should have less than 3 white blood cells per high power field (WBC/hpf). Evidence of pyuria may indicate either infection or inflammation that is not associated with bacteria. Patients that are immunosuppressed (hyperadrenocorticism, diabetes mellitus or immunosuppressive therapy) may have no evidence of inflammation, due to an inability to mount an adequate inflammatory response. Ihrke et al. (1985) found that only 54% of dogs receiving corticosteroids for the treatment of chronic skin disease had more than 3 WBC/hpf in infected urine. Evaluating white blood cells using the dipstick method alone is unreliable. Hematuria is diagnosed when a urine sample collected by cystocentesis has greater than 3 RBC/hpf. Hematuria in the absence of pyuria may be iatrogenic (due to cystocentesis) or may be due to renal or bladder wall hemorrhage that is unrelated to inflammation or infection. The visual detection of bacteria in uncontaminated urine collected by cystocentesis is diagnostic for a bacterial UTI; however, the absence of bacteria does not rule out a UTI. Rod-shaped bacteria may not be detected during examination of urine sediment if they are at a concentration <10,000/ml; cocci may not be seen if there are <100,000/ml. If the urine is dilute (specific gravity <1.013), bacteria may not be detected and white blood cell counts may not be elevated.

Infection of the urine with urea-splitting bacteria such as *Proteus* spp. or *Staphylococcus* spp. may result in an increase in urine pH (>7.5), predisposing the patient to the development of struvite urolithiasis. Struvite crystals, an alkaline pH, and evidence of urinary tract inflammation are significant findings, and should be pursued further with a urine culture and radiography or ultrasound to determine the presence of urolithiasis. Fungal elements are rarely seen during microscopic evaluation of urine sediment, but may be indicative of systemic mycotic infection. *Candida* spp.,

normally present on the external genital mucosa, may become opportunistic pathogens in immunosuppressed patients (Pressler, 2003).

Urine Culture

Urine culture is the "gold standard" for the diagnosis of urinary tract infection. Indications for performing urine culture include the detection of bacteria during urine sediment examination, evidence of pyuria, dilute urine (<1.013), immunosuppression, and diabetes mellitus or hyperadrenocorticism. Urine should be collected for culture prior to the institution of antibiotic therapy. Qualitative urine culture will identify the species of bacteria; however, it will not determine the number of bacteria. Urine culture results must be interpreted in light of the method of collection. Quantitative urine culture determines the number of bacteria per unit volume, identifies the bacterial species, and is the preferred method of culture for urine collected by any method. Techniques of urine culture have been previously reported (Ling, 1993; Osborne, 1995a). In general, urine may be sent to a diagnostic laboratory for this service or may be cultured in-house. Urine for culture should be processed within 15 minutes, but can be refrigerated for up to 6 hours or stored in tubes containing preservative without affecting bacterial growth. Susceptibility testing should be considered with complicated or recurrent cases of UTI, immunosuppressed patients, patients that have been recently catheterized, or patients treated with antimicrobials within the preceding 3 weeks (due to selection for resistance). In addition, culture and susceptibility testing should be performed in cases that do not respond within 7 days of therapy for UTI or cases that are associated with multiple pathogens.

Treatment

A detailed history will aid the clinician in determining whether the UTI is simple or complicated. A simple urinary tract infection is usually due to a transient and reversible abnormality in the host defenses, responds quickly to appropriate therapy, and does not recur. A complicated UTI is usually due to an underlying abnormality in the urinary tract or host defenses. Immunosuppression caused by glucocorticoids or other immunosuppressive drugs, hyperadrenocorticism, renal failure, or diabetes mellitus may be a cause of complicated UTI. In addition, conditions that damage the urothelium such as urolithiasis, neoplasia, catheteriza-

tion, surgery, or cystitis caused by cyclophosphamide or idiopathic causes can predispose to the development of complicated UTI. Other causes of complicated UTI include anatomic defects (ectopic ureters, urachal diverticula), interference of normal micturition (urinary obstruction, damaged innervation causing bladder atony) or changes in urine concentration or composition (glucosuria). If such an abnormality exists, UTI may relapse or recur. A relapse indicates that previous therapy failed. Reinfection is defined as a UTI that is recurrent but caused by a different bacterial organism than that previously isolated. Patients that experience a relapse or reinfection warrant further evaluation.

Treatment for a simple UTI may be empirical, based upon knowledge of the commonly isolated organisms and their susceptibility to antimicrobial agents; however, empirical therapy often fails and is not recommended (Ling, 1993). To effectively treat and eliminate a complicated UTI, further diagnostics should be considered to identify the underlying problem.

The choice of an antimicrobial to treat any infection should be based upon the ability of the drug to reach the site of infection, knowledge of side effects and adverse reactions, route of elimination, ease of administration and cost. Urine concentrations of antimicrobials are more important than serum levels during the treatment of UTI and have been previously published. In general, the urine concentration of an antibiotic will exceed that in serum if the antimicrobial is excreted in an active form in the urine. If the urine concentration of an antibiotic is four times (or more) the minimum inhibitory concentration (MIC), it will most likely be effective for treatment of UTI caused by that pathogen (90% effective) (Ling, 1993).

Knowledge of the specific bacterial species, the MIC, and of anticipated urine concentration of the antimicrobial allows the clinician to make an appropriate decision regarding antimicrobial therapy for UTI (Table 22.1). For example, since the MIC of virtually all urinary *Streptococcus* spp. and *Staphylococcus* spp. is ≤ 10 $\mu\text{g/ml}$, and 8-hour urine concentration of oral ampicillin and amoxicillin is about 300 $\mu\text{g/ml}$ at recommended doses, these drugs will be effective in the treatment of these infections, despite MICs which would be interpreted as resistant if these infections were in other sites. For the same reason, oral tetracycline has been used successfully in the treatment of urinary tract infections caused by *Pseudomonas aerug-*

inosa and cephalexin in the treatment of *Klebsiella* urinary tract infections, despite apparent "resistance" assessed in vitro using usual interpretive criteria (Ling, 1993).

The dosage and drug interval determined to treat UTI should be those recommended by the manufacturer. Ideally, three equal doses of antimicrobial per day should be given except when administering trimethoprim-sulfa or a fluoroquinolone. Most antimicrobials used to treat UTI have relatively short half-lives and may not maintain high concentrations in the urine. Therefore, client compliance during therapy for UTI is imperative (Chapter 29). In addition, some dogs void frequently during the day and may not maintain high urine concentrations of antimicrobials. It is also recommended that dogs void just prior to receiving a dose of antibiotic, especially if a long period of confinement (overnight) is anticipated. Because many drugs are excreted by the kidneys and some are nephrotoxic, patients with renal failure or insufficiency should have the dosage and interval of the chosen antibiotic adjusted (Osborne, 1995b) (Chapter 4).

In general, the duration of therapy for UTI is based upon whether the infection is simple or complicated. Single-dose therapy and three-day therapy are not effective for the treatment of experimentally induced UTI in female and male dogs, respectively (Turnwald et al., 1986; Rogers et al., 1988). Although clinical studies evaluating the optimum duration of therapy have not been done, most patients with simple UTI will require a course of antibiotics 10 to 14 days in duration. This length of treatment allows host defenses to recover and ensures the urine is sterile.

Patients with complicated UTI usually require a longer course of therapy. If an underlying problem such as neoplasia or urolithiasis is diagnosed, antimicrobial therapy is administered in conjunction with treatment of the primary problem. Many chronic, complicated cases of UTI, pyelonephritis, and prostatitis are treated with antimicrobials for four to six weeks. In many cases, it is ideal to culture the urine (via cystocentesis) one week after therapy begins and one week after it ends (urinalysis is not required) to document resolution of UTI.

In patients that have frequent recurrences of UTI without an underlying problem, prophylactic low-dose therapy can be instituted. In these patients, conventional therapy for UTI should be administered (simple or complicated), the urine should culture neg-

ative near the end of therapy, and the patient should be put on a dose that is one half to one third the total daily treatment dose. This dose should be administered once daily, prior to a period of confinement (overnight). This treatment regime should be continued for six months with monthly urine cultures collected via cystocentesis. If the cultures are negative for six months, therapy can be discontinued; however, the urine should be cultured periodically (every three to four months) to monitor for recurrence of UTI. Disadvantages of this form of therapy include the possible toxicity associated with certain antimicrobials (e.g., chloramphenicol, trimethoprim-sulfa), cost, and the risk of inducing antimicrobial resistance.

Treatment failures may be due to poor owner compliance, inappropriate choice of antimicrobial, inappropriate dose or duration of treatment, antimicrobial resistance, superinfection, or an underlying predisposing cause (e.g., urolithiasis, neoplasia, urachal diverticula). If treatment for a simple or complicated UTI fails, a complete urinalysis should be performed, including culture and susceptibility, with urine collected via cystocentesis. Plain radiographs, contrast studies, or ultrasound may be indicated in these cases. In addition, a complete blood count and biochemical profile should be performed to identify systemic disease.

Measures to prevent iatrogenic UTI include using urinary catheters only if absolutely necessary, placing them using careful aseptic technique, and using a closed-collection technique for indwelling urinary catheters. Immunosuppressed patients should be considered at increased risk for iatrogenic UTI during urinary catheterization. Results of bacterial culture of urinary catheter tips should not be used to predict whether dogs developed catheter-associated UTI (Smarick et al., 2004).

Acquired resistance to antimicrobials is of great concern for the patient, the hospital, and the community (Prescott et al., 2002). An increase in the occurrence of enrofloxacin-resistant *E. coli* has been reported, and was not attributable to a single enrofloxacin-resistant clone. Rather, there appeared to be a collective increase in enrofloxacin resistance among uropathogenic *E. coli* in dogs in general (Cooke et al., 2002). Increasing enrofloxacin resistance has also been reported in *Proteus mirabilis* and *Staphylococcus intermedius* isolates (Cohn, 2003). Multiple drug resistance in *E. coli* isolated from urine of dogs has also been reported (Sanchez et al., 2002). However,

data suggests that dogs are unlikely to be an important reservoir of antimicrobial-resistant *E. coli* strains causing infections in humans (Sannes et al., 2004). Enterococci isolated from canine UTIs have been associated with several different resistant phenotypes, with the majority exhibiting resistance to three or more antimicrobials. One *E. faecium* isolate displayed high-level resistance to vancomycin and gentamicin. Sequence analysis suggested that resistance was due to gene exchange between human and canine enterococci (Simjee et al., 2002).

Prostatitis

Prostatitis is usually caused by the ascent of normal distal urethral flora in the intact dog. This condition is rare in neutered dogs; however, an infection present prior to castration may persist post-operatively. Prostatitis may manifest as an acute or a chronic problem. The clinical signs of acute bacterial prostatitis may include depression, fever, vomiting, inappetence, urethral discharge (possibly bloody) and pain on rectal palpation of the prostate. Dogs with prostatitis may have a stiff gait and a hunched appearance. The prostate may be symmetrical and of normal size in some cases. Chronic prostatitis may be difficult to diagnose, since dogs with chronic prostatitis often do not display clinical signs other than mild inappetence and lethargy. The prostate may not be enlarged on rectal palpation, and a mild urethral discharge may or may not be present. Chronic prostatitis may be a cause or a result of chronic UTI and may lead to abscessation of the prostate.

Laboratory evaluation in the dog with acute prostatitis will often reveal neutrophilia with a left shift and a urinalysis consistent with inflammation. Patients with chronic prostatitis may or may not have changes indicative of infection on routine blood work. Urinalysis will again reveal inflammation. Radiography and ultrasonography are often useful in assisting the diagnosis of benign prostatic hypertrophy, prostatitis, prostatic cysts/abscessation, or neoplasia. Prostatic fluid evaluation is not routinely performed in the dog with suspected prostatitis.

Treatment of prostatitis should be approached in a similar manner as for a complicated UTI. Urine culture (obtained by cystocentesis or fine needle aspiration of the prostate) and sensitivity are essential in formulating a therapeutic plan. The choice of antimicrobial drug should be based upon sensitivity results

and the ability of the antimicrobial to cross the blood-prostate barrier. In cases of acute inflammation, most antimicrobials are effective; however, as the infection becomes chronic, only antimicrobials that are lipid soluble, have a basic pH, and are not highly protein-bound will cross the prostatic epithelium. Trimethoprim-sulfonamides and the fluoroquinolones have been recommended for treatment of prostatitis, but because the duration of treatment increases the risk of keratoconjunctivitis sicca from trimethoprim-sulfonamide therapy, the fluoroquinolones are the first choice. The duration of treatment should be four weeks for acute infections and six weeks for chronic infections. The urine should be cultured during therapy and recultured (by cystocentesis) one week after therapy is completed to ensure resolution of the infection. Castration may be beneficial in resolving prostatitis and preventing recurrence. Prostatic abscesses may develop as a consequence of prostatitis. They are difficult to treat medically and should be managed surgically.

Bibliography

- Bailliff NL, et al. 2005. *Corynebacterium urealyticum* urinary tract infection in dogs and cats: 7 cases (1996–2003). *J Am Vet Med Assoc* 226:1676.
- Barsanti JA, et al. 1992. Effect of therapy on susceptibility to urinary tract infection in male cats with indwelling urethral catheters. *J Vet Intern Med* 6:64.
- Buffington CAT, et al. 1997. Clinical evaluation of cats with nonobstructive urinary tract diseases. *J Am Vet Med Assoc* 210:46.
- Chastain CB, et al. 2003. Occult urinary cystitis in diabetic dogs. *Sm Anim Clin Endocrinol* 13:25.
- Cohn LA, et al. 2003. Trends in fluoroquinolone resistance of bacteria isolated from canine urinary tracts. *J Vet Diagn Invest* 15:338.
- Comer KM, Ling GV. 1981. Results of urinalysis and bacterial culture of canine urine obtained by antepubic cystocentesis, catheterization and midstream voided methods. *J Am Vet Med Assoc* 179:891.
- Cooke CL, et al. 2002. Enrofloxacin resistance in *Escherichia coli* isolated from dogs with urinary tract infections. *J Am Vet Med Assoc* 220:190.
- Drazenovich N, et al. 2004. Molecular investigation of *Escherichia coli* strains associated with apparently persistent urinary tract infection in dogs. *J Vet Intern Med* 18:301.
- Gaastera W, et al. 1996. Isolation and characterization of dog uropathogenic *Proteus mirabilis* strains. *Vet Microbiol* 48:57.
- Hostutler RA, et al. 2005. Recent concepts in feline lower urinary tract disease. *Vet Clin Small Anim* 35:147.
- Ihrke PJ, et al. 1985. Urinary tract infection associated with long-term corticosteroid administration in dogs with chronic skin diseases. *J Am Vet Med Assoc* 186:43.
- Jang SS, et al. 1984. Mycoplasma as a cause of canine urinary tract infections. *J Am Vet Med Assoc* 185:45.
- Johnson JR, et al. 2001. Phylogenetic and pathotypic similarities between *Escherichia coli* isolates from urinary tract infections in dogs and extraintestinal infections in humans. *J Infect Dis* 183:897.
- Johnson JR, et al. 2003. Identification of urovirulence traits in *Escherichia coli* by comparison of urinary and rectal *E. coli* isolates from dogs with urinary tract infection. *J Clin Microbiol* 41:337.
- Ling GV. 1993. Urinary tract infections. In: Prescott JF, Baggot JD, eds. *Antimicrobial Therapy in Veterinary Medicine*, 2nd ed. Ames: Iowa State Univ Press, p349.
- Ling GV, et al. 2001. Interrelations of organism prevalence, specimen collection method, and host age, sex, and breed among 8,354 canine urinary tract infections (1969–1995). *J Vet Intern Med* 15:341.
- Low DA, et al. 1988. Isolation and comparison of *E. coli* strains from canine and human patients with urinary tract infection. *Infect Immun* 56:2601.
- Martinez I, et al. 2003. Polypoid cystitis in 17 dogs (1978–2001). *J Vet Intern Med* 17:499.
- McGuire NC, et al. 2002. Detection of occult urinary tract infections in dogs with diabetes mellitus. *J Am Anim Hosp Assoc* 38:541.
- Norris CR, et al. 2000. Recurrent and persistent urinary tract infections in dogs: 383 cases (1969–1995). *J Am Anim Hosp Assoc* 36:484.
- Osborne CA. 1995a. Three steps to effective management of bacterial urinary tract infections: diagnosis, diagnosis, and diagnosis. *Comp Contin Educ Pract Vet* 17:1233.
- Osborne CA. 1995b. Chronic renal failure. In: Ettinger SJ, Feldman EC, eds. *Textbook of Veterinary Internal Medicine*, 3rd ed. Philadelphia: W.B. Saunders Co, 1746–1748.
- Padilla J, et al. 1981. Effects of storage time and temperature on quantitative culture of canine urine. *J Am Vet Med Assoc* 178:1077.
- Prescott JF, et al. 2002. Antimicrobial drug use and resistance in dogs. *Can Vet J* 43:107.
- Pressler BM, et al. 2003. *Candida* spp. urinary tract infections in 13 dogs and seven cats: predisposing factors, treatment, and outcome. *J Am Anim Hosp Assoc* 39:263.
- Rogers KS, et al. 1988. Effects of single-dose and three-day trimethoprim-sulfadiazine and amikacin treatment of induced *Escherichia coli* urinary tract infections in dogs. *Am J Vet Res* 49:345.
- Sanchez S, et al. 2002. Characterization of multidrug-resistant *Escherichia coli* isolates associated with nosocomial infections in dogs. *J Clin Microbiol* 40:3586.
- Sannes MR, et al. Antimicrobial resistance of *Escherichia coli* strains isolated from urine of women with cystitis or pyelonephritis and feces of dogs and healthy humans. *J Am Vet Med Assoc* 225:368.
- Seguin MA, et al. 2003. Persistent urinary tract infections and reinfections in 100 dogs. *J Vet Intern Med* 17:622.

- Senior DF, et al. 1992. Serotype, hemolysin production, and adherence characteristics of strains of *E. coli* causing urinary tract infection in dogs. *Am J Vet Res* 53:4.
- Simjee S, et al. 2002. Characterization of Tn1546 in vancomycin-resistant *Enterococcus faecium* isolated from canine urinary tract infections: evidence of gene exchange between human and animal enterococci. *J Clin Microbiol* 40:4659.
- Smarick SD, et al. 2004. Incidence of catheter-associated urinary tract infection among dogs in a small animal intensive care unit. *J Am Vet Med Assoc* 224:1936.
- Starcic M, et al. 2002. Haemolytic *Escherichia coli* isolated from dogs with diarrhea have characteristics of both uropathogenic and necrotoxic strains. *Vet Microbiol* 85:361.
- Stiffler KS, et al. 2003. Clinical use of low-profile cystotomy tubes in four dogs and a cat. *J Am Vet Med Assoc* 223:325.
- Swenson CL, et al. 2004. Evaluation of modified Wright-staining of urine sediment as a method for accurate detection of bacteriuria in dogs. *J Am Vet Med Assoc* 224:1282.
- Torres SM, et al. 2005. Frequency of urinary tract infection among dogs with pruritic disorders receiving long-term glucocorticoid treatment. *J Am Vet Med Assoc* 227:239.
- Turnwald GH, et al. 1986. Comparison of single-dose and conventional trimethoprim-sulfadiazine therapy in experimental *Staphylococcus intermedius* cystitis in the female dog. *Am J Vet Res* 47:2621.
- Wilson RA, et al. 1988. Strains of *E. coli* associated with urogenital disease in dogs and cats. *Am J Vet Res* 49:743.

Antimicrobial Therapy of Selected Bacterial Infections

John F. Prescott

This chapter discusses special considerations required when treating selected bacterial infections (anaerobic, atypical mycobacterial, *Brucella*, leptospirosis, mycoplasma, and *Nocardia*).

Anaerobic Infections

Obligate anaerobic bacteria (anaerobes) are those that are unable to grow in the presence of molecular oxygen. They can be Gram-negative or Gram-positive rods or cocci, and comprise a significant proportion of bacterial populations that comprise the normal flora on all the mucosal surfaces that harbor bacteria. Only a few of the several hundred different species produce primary disease. These exceptions include members of the genera *Clostridium* (example, *C. difficile*, *C. perfringens*), enterotoxigenic *Bacteroides fragilis*, and the pathogenic anaerobic spirochetes (examples, *Brachyspira*, *Serpulina*). The great majority of other anaerobes that cause disease in animals are opportunistic pathogens. The most commonly encountered infectious processes involving anaerobes are those stemming from inoculation (infection) of a normally sterile site by a member of the relatively pathogenic species of the genera of normal flora (*Actinomyces*, *Bacteroides*, *Clostridium*, *Eubacterium*, *Peptostreptococcus*, *Porphyromonas*, etc.) occupying the mucosal surface contiguous to the compromised site.

Resistance

All anaerobes are naturally resistant to the aminoglycosides, since these antibiotics require an oxygen-dependant transport system to get into the bacterial cell. Likewise, anaerobes are inherently resistant to the earlier fluoroquinolones, though some newer ones have activity against some anaerobes (levofloxacin,

grepafloxacin, trovafloxacin) (Goldstein, 1996). Moxifloxacin was found to be effective against 83% of a large number of human clinical isolates (Goldstein et al., 2006), suggesting its possible use in monotherapy against some community-acquired intra-abdominal infections.

Some anaerobes are resistant to cell-wall-active antimicrobials—the penicillins and first-generation cephalosporins—because of genes encoding enzymes that break down these drugs (Stark et al., 1993; Sebald, 1994). The most commonly encountered resistance gene encodes a cephalosporinase that is active on the first-generation cephalosporins, the penam penicillins, and some third-generation cephalosporins (notably ceftiofur) (Livermore, 1995; Samitz et al., 1996). Members of the *Bacteroides fragilis* group (*B. distasonis*, *B. eggerthii*, *B. fragilis*, *B. sterocoralis*, *B. ovatus*, and *B. uniformis*) more often than not contain the gene encoding this enzyme. Much of the data on resistance comes from human rather than veterinary sources (Falagas and Siakavella, 2000), but the findings are probably reasonably applicable to animals.

Resistance to the tetracyclines is unpredictable because of acquired resistance. The effectiveness of trimethoprim-sulfonamides is also unpredictable for the treatment of infectious processes involving anaerobes. This is because some anaerobes (and there is no way to predict which) are able to scavenge thymidine from necrotic material and thereby bypass the block in the production of this chemical by trimethoprim-sulfonamides (Indiveri and Hirsh, 1992). So even though in vitro tests (done under controlled thymidine-free conditions) predict effectiveness in vivo, trimethoprim-sulfonamide combinations are not recommended for treatment of infectious processes involving anaerobes.

In vitro susceptibility testing of anaerobic bacteria has not been standardized and there appears to be variability between laboratory media, methods and interpretive criteria. The E-test (Chapter 2) may represent a simple approach to testing for a limited (because of high cost) range of drugs.

Susceptibility

Chloramphenicol, the macrolides, metronidazole, clindamycin, and some second- (cefoxitin) and third-generation cephalosporins (ceftizoxime) are effective in the treatment of anaerobic infections (Jang et al., 1997). Penicillins (penicillin G, amoxicillin, ampicillin, ticarcillin) are effective against most anaerobes (except members of the *B. fragilis* group and occasionally other Gram-negative species), but when combined with clavulanic acid (which irreversibly binds the cephalosporinase produced by resistant strains), beta-lactams are effective against virtually all anaerobes (Appelbaum et al., 1990).

Resistance to metronidazole and to clindamycin is uncommon; thus, these are often drugs of choice for treating anaerobic infections (Jang et al., 1997a). An isolated report of clinically significant metronidazole resistance has been reported for *C. difficile*-associated diarrhea affecting horses in a teaching hospital (Jang et al., 1997b).

Clinical Application

Infectious processes involving normally sterile sites are usually a mixture of anaerobes and aerobes (facultative as well as obligate species). Many anaerobic bacterial infections are mixed, but attempted elimination of all the organisms may not be necessary. This is because the unique synergism that sometimes occurs between aerobic and anaerobic bacteria is such that elimination of only some of the species present in the mixed bacterial infection will result in removal of the synergistic effect and clearance of the infection. Treatment of anaerobic infections should, wherever possible, include drainage and debridement. For example, in cat bite infections, debridement, drainage, and removal of *Pasteurella multocida* and a proportion of the anaerobic bacterial species by ampicillin or amoxicillin is usually adequate to resolve the infection.

In human medicine, no consensus has been reached regarding the specific agents, dosage and duration of therapy of anaerobic infections (Falagas and Siakavella, 2000), so that clinical judgment is required in

making these choices. Whether the veterinary clinician chooses an antimicrobial combination or a single antimicrobial drug will depend on assessment of the seriousness of the infection and of its consequences.

Empirical treatment (usually the case since susceptibility test results of aerobic organisms are unavailable for at least 48 hours, and of anaerobic species, at least 5 days) of such conditions is relatively straightforward. The severity of the infection will be one factor dictating the choice of antimicrobial drugs (Table 23.1). For serious infectious, a combination of drugs effective against the aerobic component with drugs effective against the anaerobic component may be chosen. Examples include an aminoglycoside or a fluoroquinolone with amoxicillin-clavulanic acid (Jang et al., 1997a). One example of the use of such combinations is the treatment of intra-abdominal infections involving spillage of large bowel contents into the intestine. This represents a very serious condition that needs prevention of the likely aerobic bacterial (*E. coli*) peritonitis and the subsequent development of abscesses due to anaerobic bacteria (Goldstein and Snyderman, 2004). Septic pleuritis in horses is another condition in which it is usual to combine treatment against the aerobic (*E. coli* and especially *Streptococcus equi* subsp. *zooepidemicus*) component and the likely nonsporeforming anaerobic bacterial component that may be a consequence of the infection. One typical combination is penicillin-gentamicin for the aerobes and metronidazole for the anaerobes (Wilkins, 2003).

Treatment of anaerobic intestinal infections (enterotoxigenic *B. fragilis*, *Brachyspira hyodysenteriae*, *B. pilosicoli*, *C. difficile*, *C. perfringens*) involves a range of choices. Diarrhea associated with *C. perfringens* responds to treatment with ampicillin (amoxicillin), erythromycin, tylosin, or metronidazole. Diarrhea associated with *C. difficile* responds to metronidazole although rare horses may be affected with metronidazole-resistant strains (Jang et al., 1997b; Magdesian et al., 1997). Treatment of *B. pilosicoli* and enterotoxigenic *B. fragilis*-associated diarrhea is with metronidazole. Treatment of disease produced by *B. hyodysenteriae* is discussed in Chapter 34.

Bibliography

- Appelbaum PC, et al. 1990. Beta-lactamase production and susceptibilities to amoxicillin, amoxicillin-clavulanate, ticarcillin, ticarcillin-clavulanate, cefoxitin, imipenem and

Table 23.1. Choice of antimicrobial drugs to treat nonsporeforming anaerobic infections in animals.

Type of infection	Single agent	Combination of agents
Relatively non-serious, e.g., bite infections	Amoxicillin, ampicillin, azithromycin, chloramphenicol, clindamycin, erythromycin, tylosin	Amoxicillin-clavulanic acid, sulbactam-ampicillin
Serious infections, including intra-abdominal infections	Cefoxitin	Amoxicillin-clavulanic acid; piperacillin-tazobactam; sulbactam-ampicillin; ticarcillin-clavulanic acid; aminoglycoside (amikacin, gentamicin, tobramycin) plus metronidazole or clindamycin; 3rd/4th-generation cephalosporin plus metronidazole or clindamycin; fluoroquinolone plus metronidazole
Very serious infections (valuable animal)	Meropenem	Imipenem-cilastatin

metronidazole of 320 non-*Bacteroides fragilis* *Bacteroides* isolates and 129 *Fusobacterium* from 28 U.S. centers. Antimicrob Agents Chemother 34:1546.

Falagas ME, Siakavella E. 2000. *Bacteroides*, *Prevotella*, and *Porphyromonas* species: a review of antibiotic resistance and therapeutic options. Int J Antimicrob Agents 15:1.

Goldstein EJC. 1996. Possible role for the new fluoroquinolones (levofloxacin, grepafloxacin, trovafloxacin, clinafloxacin, sparfloxacin, and DU-6859a) in the treatment of anaerobic infections: review of current information on efficacy and safety. Clin Infect Dis 23:S25.

Goldstein EJC, Snyderman. 2004. Intra-abdominal infections: review of the bacteriology, antimicrobial susceptibility and the role of ertapenem in their therapy. J Antimicrob Chemother 53:SupplS2,ii29.

Goldstein EJC, et al. 2006. In vitro activity of moxifloxacin against 923 anaerobes isolated from human intra-abdominal infections. Antimicrob Agents Chemother 50:148.

Indiveri MC, Hirsh DC. 1992. Tissues and exudates contain sufficient thymidine for growth of anaerobic bacteria in the presence of inhibitory levels of trimethoprim-sulfamethoxazole. Vet Microbiol 32:235.

Jang SS, et al. 1997a. Organisms isolated from dogs and cats with anaerobic infections and susceptibility to selected antimicrobial agents. J Am Vet Med Assoc 210:1610.

Jang SS, et al. 1997b. Antimicrobial susceptibilities of equine isolates of *Clostridium difficile* and molecular characterization of metronidazole-resistant strains. Clin Infect Dis 25:Suppl. 2:S266.

Livermore DM. 1995. Beta-lactamases in laboratory and clinical resistance. Clin Microbiol Rev 8:557.

Madewell BR, et al. 1995. Apparent outbreak of *Clostridium difficile*-associated diarrhea in horses in a veterinary medical teaching hospital. J Vet Diagn Invest 7:343.

Magdesian KG, et al. 1997. *Clostridium difficile* and horses: a review. Rev Med Microbiol 8:S46.

Samitz EM, et al. 1996. In vitro susceptibilities of selected obligate anaerobic bacteria obtained from bovine and equine sources to ceftiofur. J Vet Diagn Invest 8:121.

Wexler HM, Finegold SM. 1998. Current susceptibility patterns of anaerobic bacteria. Yonsei Med J 39:495.

Wilkins PA. 2003. Lower airway diseases of the adult horse. In Parente EJ, ed. Respiratory disease. Vet Clin North Am Equine Pract 19:101.

Brucella

Brucellosis is the disease produced by members of the genus *Brucella*. The genus contains eight species: *B. abortus*, *B. canis*, *B. cetaceae*, *B. melitensis*, *B. neotomae*, *B. ovis*, *B. pinnipediae*, and *B. suis*. Treatment of brucellosis is usually restricted to affected companion animals, i.e., dogs and horses, because the disease in food-producing livestock is controlled by national eradication programs. Treatment strategies are expensive and involve long-term administration of antibiotics that may not be approved for use in food-producing animals. Brucellae are facultative intracellular parasites that survive within macrophages. This fact is important in predicting in vivo efficacy when using the results of in vitro susceptibility tests. Therapy with two antimicrobials is indicated because of recurrence of disease after cessation of single antimicrobial therapy (Solera et al., 1997). Experimental evidence and clinical experience treating human patients have shown that at least one of the antibiotics should have intracellular distribution (Solera et al., 1997).

Though very susceptible in vitro, relapses are common with monotherapy with the tetracyclines, rifampins, and trimethoprim-sulfonamides (Solera et al., 1997). Brucellae are also very susceptible in vitro to the fluoroquinolones, but clinical data show that treatment of human patients with ciprofloxacin alone is ineffective, perhaps because fluoroquinolones are less active at the acid pH of the phagolysosome (García-Rodríguez et al., 1991). However, combination of a

fluroquinolone with rifampin had an 85% cure rate in a small group of human patients (Agalar et al., 1999). A critical review of the literature concluded that use of quinolones alone is associated with unacceptably high rates of relapse and, when used in combination with rifampin or doxycycline, does not lead to improved outcomes over those associated with conventional regimens (Falagas and Bliziotis, 2006).

The treatments that have been found to control brucellosis in human patients involve the use of two agents: doxycycline plus an aminoglycoside (e.g., gentamicin) or doxycycline plus rifampin are synergistic combinations (Solera et al., 1997). For children, because of the tooth-staining effects of tetracyclines, rifampin plus trimethoprim-sulfonamide or rifampin plus an aminoglycoside are recommended alternatives (Solera et al., 1997). Therapies showing promise (effective in rodent models of brucellosis) include the newer macrolides clarithromycin or azithromycin, and liposomal formulations containing an aminoglycoside (gentamicin) (Hernández-Caselles et al., 1989; Lang et al., 1994). In the latter case, high intracellular concentrations of this formulation of drug are attained. Since brucellae have zoonotic potential, careful consideration should be given to the appropriateness of treatment. Recommended treatment regimes also include a tetracycline for four weeks with an aminoglycoside during weeks 1 and 4 (Carmichael and Greene, 1998). There are no current published recommendations for a tetracycline and rifampin for animals with brucellosis, but clinical data acquired from human experience indicate that tetracycline plus rifampin should be given together for at least six weeks.

Bibliography

- Carmichael LE, Greene CE. 1998. Canine brucellosis. In: Greene CE (ed). *Infectious Diseases of the Dog and Cat*, 2nd ed. Philadelphia: WB Saunders, p248.
- Falagas ME, Bliziotis IA. 2006. Quinolones for treatment of human brucellosis: Critical review of the evidence from microbiological and clinical studies. *Antimicrob Agents Chemother* 50:22.
- García-Rodríguez JA, et al. 1991. Lack of effective bactericidal activity of new quinolones against *Brucella* spp. *Antimicrob Agents Chemother* 35:756.
- Hernández-Caselles T, et al. 1989. Treatment of *Brucella melitensis* infection in mice by use of liposome-encapsulated gentamicin. *Am J Vet Res* 50:1486.
- Lang R, et al. 1994. Therapeutic effects of roxithromycin and azithromycin in experimental murine brucellosis. *Chemother* 40:252.
- Pappas G, et al. 2005. New approaches to the antibiotic treatment of brucellosis. *Int J Antimicrob Agents* 26:101.
- Solera J, et al. 1997. Recognition and optimum treatment of brucellosis. *Drugs* 53:245.

Atypical Mycobacteria

For convenience, members of the genus *Mycobacterium* are categorized into those that produce tuberculosis (*M. tuberculosis*, *M. bovis*), leprosy (*M. leprae*), and the atypical mycobacteria. The atypical mycobacteria are composed of those species that are so-called slow growers (taking weeks to months to form visible colonies in vitro: e.g., *M. avium* complex, *M. genavense*, *M. goodii*, *M. kansasii*, *M. marinum*, *M. simiae*, *M. szulgai*, *M. ulcerans*, and *M. xenopi*) and those that are called rapid growers (days to weeks to form visible colonies in vitro: e.g., *M. chelonae*, *M. fortuitum*, *M. phlei*, *M. smegmatis*, and *M. vaccae*). The distinction between rapid growers and slow growers is sometimes important when trying to formulate a treatment strategy since there are differences in susceptibility between these two groups (García-Rodríguez and García, 1993).

Members of the *M. avium* complex are the main atypical mycobacteria affecting human patients with acquired immunodeficiency syndrome. *M. avium* also affects birds (second to *M. genavense* in pet birds), swine, and rarely, horses and sheep. Dogs and cats are highly resistant to disease caused by members of the *M. avium* complex (though disseminated disease has been described in previously normal cats), being affected most often by other atypical strains such as *M. chelonae*, *M. fortuitum*, *M. lepraemurium* (cats), *M. phlei*, *M. smegmatis*, and *M. xenopi*. Almost all of the atypical mycobacteria are environmental dwellers, and as such the environment is the major source of infection, rather than an infected patient (Heifets, 1996). Some form of immunosuppression is usually a prerequisite for disease.

Numerous trials involving human patients have demonstrated that monotherapy leads to the development of resistance to the drug being used (Heifets, 1996; Alangaden and Lerner, 1997). Consequently, most regimens recommended for the treatment of atypical mycobacteriosis involve the use of at least two antimicro-

bial drugs. In addition, mycobacteria are facultative intracellular parasites, able to survive within the phagolysosome. Thus it is important when choosing an antibiotic that drugs be used that penetrate into cells.

Resistance

Mycobacteria are naturally resistant to all of the antibiotics that affect the cell wall (penicillins and cephalosporins), probably because of the high lipid content of the mycobacterial cell wall. Resistance rapidly occurs subsequent to use of a single antimicrobial to which the bacterium was originally susceptible. Resistance results from mutations in the chromosomal gene encoding the target of the antibiotic. However, sulfonamide resistance has been found on a transposable element in an isolate of *M. fortuitum*, the only example of this occurrence (Zhang and Young, 1994).

Susceptibility

There are no firm rules for treating infectious processes that involve atypical mycobacteria. The following recommendations have been taken in part from recommendations for treating human patients and from the few successful attempts at treating animals. In general, the antibiotics used to treat typical mycobacterial infections (caused by *M. bovis* or *M. tuberculosis*) are ineffective for infectious processes involving atypical mycobacteria (Davis et al., 1987). Most strains of atypical mycobacteria are susceptible to clarithromycin (and azithromycin). Drugs that have shown effectiveness as added partners to clarithromycin include: clofazimine; fluoroquinolones (members of the *M. avium* complex are unpredictable, most human isolates are susceptible, most animal isolates are resistant, and *M. chelonae* is resistant); ethambutol (*M. fortuitum* resistant); rifampin (*M. fortuitum* resistant); amikacin (most predictably active against rapid growers) (Khardori et al., 1994; Kaufman et al., 1995; Heifets, 1996; Yajko et al., 1996; Alangaden and Lerner, 1997; Watt, 1997).

Clinical Application

The first clues that an atypical mycobacterium may be involved is the presence of chronically occurring lesions that include draining tracts, lack of response to a variety of antimicrobial agents, and the lack of growth on media after 24–48 hours of incubation. In addition to historical clues, if portions of the affected area are

stained with either a Romanovsky-type stain (Giemsa; Wright's) or with Gram's stain, atypical mycobacterial cells have characteristic properties. Romanovsky-stained bacterial cells may appear as "ghosts", and with Gram's stain, they may appear as rods with "speckles". Such clues should prompt the use of acid-fast stain, and the inoculation of appropriate media to be incubated for a suitable length of time. If an acid-fast bacterium is present, then appropriate antibiotic therapy should be started. A combination of clarithromycin and a fluoroquinolone (and/or rifampin, ethambutol, or clofazimine) is a possible starting regimen. If an isolate is obtained, it should be sent to an appropriate reference laboratory for susceptibility testing. Treatment should involve surgical drainage wherever possible and prolonged antimicrobial therapy, which might last for months, depending on clinical response and the nature of the infection.

Bibliography

- Alangaden GJ, Lerner SA. 1997. The clinical use of fluoroquinolones for the treatment of mycobacterial diseases. *Clin Infect Dis* 25:1213.
- Byrne SK, et al. 1990. Comparison of *in vitro* antimicrobial susceptibilities of *Mycobacterium avium*-*M. intracellulare* strains from patients with acquired immunodeficiency syndrome (AIDS), patients without AIDS, and animal sources. *Antimicrob Agents Chemother* 34:1390.
- Davis CE, et al. 1987. *In vitro* susceptibility of *Mycobacterium avium* complex to antibacterial agents. *Diagn Microbiol Infect Dis* 8:149.
- Grange JM, et al. 1990. The avian tubercle bacillus and its relatives. *J Appl Bacteriol* 68:411.
- Heifets L. 1996. Susceptibility testing of *Mycobacterium avium* complex isolates. *Antimicrob Agents Chemother* 40:1759.
- Jogi R, Tying SK. 2004. Therapy of nontuberculous mycobacterial infections. *Derm Therap* 17:491.
- Kaufman AC, et al. 1995. Treatment of localized *Mycobacterium avium* complex infection with clofazimine and doxycycline in a cat. *J Am Vet Med Assoc* 207:457.
- Khardori N, et al. 1994. *In vitro* susceptibilities of rapidly growing mycobacteria to newer antimicrobial agents. *Antimicrob Agents Chemother* 38:134.
- Watt B. 1997. *In vitro* sensitivities and treatment of less common mycobacteria. *J Antimicrob Chemother* 39:567.
- Yajko DM, et al. 1996. *In vitro* activities of rifabutin, azithromycin, ciprofloxacin, clarithromycin, clofazimine, ethambutol, and amikacin in combination of two, three, and four drugs against *Mycobacterium avium*. *Antimicrob Agents Chemother* 40:743.
- Zhang Y, Young D. 1994. Molecular genetics of drug resistance in *Mycobacterium tuberculosis*. *J Antimicrob Chemother* 34:313.

Mycoplasma

The term "mycoplasma" is used to denote a member of the order Mycoplasmatales and class Mollicutes. Six genera are recognized: *Acholeplasma*, *Anaeroplasm*, *Asteroplasm*, *Mycoplasma*, *Spiroplasma* and *Ureaplasma*, but of these only members of the genera *Mycoplasma* and *Ureaplasma* are important in veterinary medicine. Mycoplasmas are associated with the respiratory tract, arthritis, mastitis, septicemia, and the urogenital tract of many animal species.

Resistance

Because of their inability to synthesize a cell wall, all mycoplasmas are resistant to all cell wall active antibiotics (penicillins, cephalosporins, glycopeptides, etc). In addition, mycoplasmas are resistant to rifampin. Some species, such as *M. bovis* and *M. hyopneumoniae*, are intrinsically resistant to 14-membered macrolides such as erythromycin. Strains of mycoplasma from farm animals are increasingly resistant to the tetracyclines, although the genetic basis of resistance of mycoplasmas to tetracyclines and other antimicrobial drugs has not been well characterized (Ter Laak et al., 1993; Rosenbusch et al., 2005; Aarestrup and Kempf, 2006). In Denmark, the progressive development of resistance to tylosin over two decades by *M. hyopneumoniae* was linked to the extensive use of this drug in swine during this period (Aarestrup and Friis, 1998). In one study, many *M. bovis* isolates were resistant to spectinomycin, tylosin and tetracycline (Thomas et al., 2003). In vitro resistance of animal-derived mycoplasma should be determined more frequently than has been done in the past, perhaps using the simplicity of the Etest assay (Francoz et al., 2005).

Susceptibility

It is difficult to easily ascertain susceptibility since in vitro testing of isolates is extremely difficult, and is usually not performed except by specialized laboratories. In general, however, the macrolides (in particular azithromycin, clarithromycin, erythromycin [usually], tylosin, tiamulin) and the fluoroquinolones appear to be the most active (Ter Laak et al., 1993; Kobayashi et al., 1996; Musser et al., 1996; Hannan et al., 1997). Mycoplasmas are also usually susceptible in vitro to aminoglycosides, chloramphenicol, lincosamides and tetracyclines (but see comment above). In poultry, injection of eggs with aminoglycosides is effective in

eliminating mycoplasmas. Ketolides (e.g., telithromycin) are highly active against mycoplasmas. With the exception of the fluoroquinolones, which are bactericidal, the bacteriostatic activity of mycoplasma-active antibiotics may be another factor that makes mycoplasma infections often only slowly responsive to treatment.

Clinical Application

Mycoplasmas are often both hard to isolate and slow-growing. As a consequence, treatment of mycoplasma infections is usually empirical rather than based on in vitro susceptibility. Elimination from tissues is, however, often slow, since most antibiotics have only a bacteriostatic effect against mycoplasma. In addition, there is increasing evidence that some mycoplasma may become intracellular (Taylor-Robinson and Bebear, 1997). For both of these reasons, treatment of established mycoplasma infections in animals is sometimes disappointing despite excellent susceptibility in vitro (Ross and Cox, 1988), perhaps because effective treatment may require 2–3 weeks rather than a shorter course. There is a paucity of data on the clinical efficacy of treatment of many mycoplasma infections in animals, which contrasts with the proven efficacy in human medicine of tetracycline or macrolide treatment of mycoplasma pneumonia (Taylor-Robinson and Bebear, 1997). The guiding general principle required for effective treatment of a mycoplasma infection is therefore to choose an antibiotic (fluoroquinolone, lincosamide, macrolide, tetracycline) which penetrates cells well and to administer the drug for 2–3 weeks, with isolation and in vitro susceptibility testing in cases of failure of clinical response.

Bibliography

- Aarestrup FM, Friis NF. 1998. Antimicrobial susceptibility testing of *Mycoplasma hyosynoviae* isolated from pigs during 1968 to 1971 and during 1995 and 1996. *Vet Microbiol* 61:33.
- Aarestrup FM, Kempf I. 2006. Mycoplasma. In Aarestrup FM, ed. *Antimicrobial Resistance in Bacteria of Animal Origin*. Washington, D.C.: ASM Press, 239-248.
- Francoz D, et al. 2005. Determination of *Mycoplasma bovis* susceptibilities against six antimicrobial agents using the Etest method. *Vet Microbiol* 105:57.
- Hannan PCT, et al. 1997. Comparative susceptibilities of various animal-pathogenic mycoplasmas to fluoroquinolones. *Antimicrob Agents Chemother* 41:2037.

- Hannan PC. 2000. Guidelines and recommendations for antimicrobial minimum inhibitory concentration (MIC) testing against veterinary mycoplasma species. International Research Programme on Comparative Mycoplasmaology. Vet Res 31:373.
- Kobayashi H, et al. 1996. Macrolide susceptibility of *Mycoplasma hyorhinis* isolated from piglets. Antimicrob Agents Chemother 40:1030.
- Musser J, et al. 1996. Comparison of tilmicosin with long-acting oxytetracycline for treatment of respiratory tract disease in calves. J Am Vet Med Assoc 208:102.
- Ross RF, Cox DF. 1988. Evaluation of tiamulin for treatment of mycoplasmal pneumonia in swine. J Am Vet Med Assoc 193:441.
- Rosenbusch RF, et al. 2005. In vitro antimicrobial inhibition profiles of *Mycoplasma bovis* isolates recovered from various regions of the United States from 2002 to 2003. J Vet Diagn Invest 17:436.
- Taylor-Robinson D, Bebear C. 1997. Antibiotic susceptibilities of mycoplasmas and treatment of mycoplasmal infections. J Antimicrob Chemother 40:622.
- Ter Laak EA, et al. 1993. Susceptibilities of *Mycoplasma bovis*, *Mycoplasma dispar*, and *Ureaplasma diversum* strains to antimicrobial agents in vitro. Antimicrob Agents Chemother 37:317.
- Thomas A, et al. 2003. Antibiotic susceptibilities of recent isolates of *Mycoplasma bovis* in Belgium. Vet Rec 153:428.

Nocardia

Nocardiosis has been reported in a variety of animal species, but of the domesticated variety, cattle, horses, dogs, and cats are most commonly affected (Beaman and Beaman, 1994). *Nocardia asteroides* is the species most frequently reported. This species is heterogeneous, being composed of *N. asteroides sensu stricto*, *N. farcinia*, and *N. nova* (Wallace et al., 1991). The distinguishing characteristics used to differentiate among these three species are the types of cell wall mycolic acids and the susceptibility to antimicrobial agents. *Nocardia nova* is the species most often isolated from dogs and cats (localized lesions are most often associated with an extremity), whereas *N. asteroides sensu stricto* is most often isolated from cattle and horses (Biberstein et al., 1985). Nocardioform placentitis, the most common cause of placentitis in mares from central Kentucky, is not caused by *Nocardia* spp. but rather by *Amycolatopsis* spp. (*A. kentuckyensis*, *A. lexingtonensis*, *A. pretoriensis*), *Crossiella equi*, or *Cellulosimicrobium cellulans* (Labeda et al., 2003; Bolin et al., 2004).

Though *N. asteroides sensu stricto* and *N. nova* re-

Table 23.2. Comparison of the susceptibility of *Nocardia asteroides sensu stricto* with *N. nova*.

Antimicrobial drug	<i>Nocardia asteroides sensu stricto</i> (% susceptible)	<i>Nocardia nova</i> (% susceptible)
Ampicillin	27	44
Amoxicillin-clavulanate	67	6
Cefuroxime, cefotaxime, ceftriaxone	94-98	Cefuroxime 100%; other 3rd-generation 83-94%
Ciprofloxacin	38	0
Dapsone	92	94
Doxycycline	88	94
Minocycline	94	100
Amikacin	90-95	100
Erythromycin	60	100
Clarithromycin	—	100
Trimethoprim-sulfa	100	89
Imipenem	77	100
Tobramycin	—	33

From Hirsh DC, 2000.

main susceptible to the sulphonamides (trimethoprim-sulfas are most commonly used), long-term treatment with this class of antimicrobial is sometimes associated with undesirable side effects (Chapter 16). Alternative treatment includes the tetracyclines (minocycline, doxycycline); some cephalosporins; and imipenem (see Table 23.2). However, if the use of other antibiotics is contemplated, then it may make a great deal of difference whether an animal is affected with *N. asteroides sensu stricto* (often resistant to macrolides and penicillins) or *N. nova* (often susceptible to macrolides and sometimes to penicillins) (Lerner, 1996).

The diagnosis of nocardiosis can be made by observation of moderately acid-fast branching filaments in a sample collected from the affected site, or by culture. Trimethoprim-sulfa drugs are the antimicrobial of choice, though depending upon animal species, other choices may be available (see above and Table 23.2).

Bibliography

- Beaman BL, Beaman L. 1994. *Nocardia* species: Host parasite relationships. Clin Microbiol Rev 7:213.
- Biberstein EL, et al. 1985. *Nocardia asteroides* infection in horses: a review. J Am Vet Med Assoc 186:273.

- Bolan DC, et al. 2004. Equine abortion and premature birth associated with *Cellulosimicrobium cellulans* infection. *J Vet Diagn Invest* 16:333.
- Hirsh DC. 2000. *Nocardia*. In Prescott JF, et al (eds): *Antimicrobial Therapy in Veterinary Medicine*, 3rd edition, Ames: Iowa State University Press: p469.
- Labeda DP, et al. 2003. *Amycolatopsis kentuckyensis* sp. nov., *Amycolatopsis lexingtonensis* sp. nov. and *Amycolatopsis pretoriensis* sp. nov., isolated from equine placentas. *Int J Syst Evol Microbiol* 53:1601.
- Lerner PI. 1996. Nocardiosis. *Clin Infect Dis* 22:891.
- Wallace RJ, et al. 1991. Clinical and laboratory features of *Nocardia nova*. *J Clin Microbiol* 29:2407.

Leptospira and Leptospirosis

MIC determinations show leptospires to be susceptible to a wide variety of antimicrobial drugs. They are highly susceptible in vitro to penicillin G, ampicillin, amoxicillin, cefotaxime, erythromycin, and fluoroquinolones. Susceptibility to streptomycin, tylosin, tiamulin, and tetracyclines is good. They are relatively resistant to cephalothin, chloramphenicol and sulfonamides. Penicillin G has weak bactericidal activity. Acquired resistance has not been reported. Experimental infections with laboratory animals have established the value of penicillin G, erythromycin, streptomycin, and tetracyclines in treatment of leptospirosis. Cephalexin, cefadroxil and cefoperazone had little activity although cefotaxime was effective. First- and second-generation cephalosporins should therefore not be used for treatment. Treatment of human patients has established the value of penicillin G and doxycycline therapy in leptospirosis.

In acute leptospirosis, recommended treatments in animals include ampicillin or amoxicillin, penicillin G, streptomycin, doxycycline or other tetracyclines, and erythromycin. Amoxicillin (or ampicillin) or doxycycline are probably the drugs of choice. Since amoxicillin is bactericidal, there may be a danger in treatment of acute leptospirosis of endotoxin release with adverse effects on the host (Jarisch-Herxheimer reaction), so that doxycycline may have a marginal advantage over amoxicillin. Treatment should probably last 7 days. It seems unnecessary to treat dogs with acute leptospirosis with amoxicillin for a week followed by doxycycline for three weeks, as recommended in some texts, and there is no data supporting such an approach. One major advantage of streptomycin, which can be combined with amoxicillin treatment, is that

the persistence of this antibiotic in the kidney after even a single injection assists the removal of the kidney carrier state. Unfortunately, streptomycin is often difficult to obtain.

Chronic leptospirosis is characterized by abortion and stillbirth, recurrent iridocyclitis, repeat breeding in pigs and possibly cattle, and subclinical meningeal infection, depending on the serovar involved and the animal species affected. Many studies of *L. pomona* infection in swine and cattle have established the value of a single IM injection of 25 mg/kg dihydrostreptomycin or streptomycin in removing the kidney carrier state. It did not, however, remove serovar *hardjo* from the genital tract and kidney of bovine carriers in one study (Ellis et al., 1985). Oral treatment of swine with tetracyclines (800 g/ton of feed for 8–11 days) will control leptospirosis but cannot be relied on to remove renal carriage, possibly because of tetracycline's bacteriostatic action. In outbreaks of leptospiral abortion in cattle, the usual recommendation is to vaccinate after treating once with streptomycin.

Since streptomycin is now prohibited in some countries for use in food animals, attempts have been made to find alternatives. Injection of one or two (q48 hours) doses of 15 mg/kg of amoxicillin was found to remove the kidney carrier state of serovar *hardjo* in cattle (Smith et al., 1997). Tylosin (44 mg/kg for 5 days), erythromycin (25 mg/kg for 5 days), and tetracycline (40 mg/kg for 3 to 5 days), all given IM q24 hours, effectively removed kidney carriage of serovar *pomona* in swine. Ceftiofur and ampicillin at standard dosage for 3–5 days was not effective (Alt and Bolin, 1996). The effective drugs listed above can all be recommended during outbreaks of abortion. Further studies are needed to determine whether and what antimicrobial treatments are effective in therapy of peridodic ophthalmia of horses.

Bibliography

- Alt DP, Bolin CA. 1996. Preliminary evaluation of antimicrobial agents for treatment of *Leptospira interrogans* serovar *pomona* infection in hamsters and swine. *Am J Vet Res* 57:59.
- Prescott JF. 1991. Treatment of leptospirosis. *Cornell Vet* 81:7.
- Smith CR, et al. 1997. Amoxycillin as an alternative to dihydrostreptomycin sulphate for treating cattle infected with *Leptospira borgpetersenii* serovar *hardjo*. *Aust Vet J* 75:818.

Growth Promotion Uses of Antimicrobial Agents

Thomas R. Shryock and Stephen W. Page

The inclusion of antimicrobial agents in the feed of food-producing animals for the purpose of enhancing physiological performance is a province of antimicrobial use that has not historically attracted the attention and professional involvement of many veterinarians. Those antimicrobial agents with growth promoting activity have generally been available without a veterinary prescription and decisions on their use have often been based on economic, nutritional and animal performance considerations, rather than on questions of disease control. However, concerns have grown in recent decades about the possibility of adverse public health impacts arising from the selection and dissemination of antimicrobial resistance from antimicrobial growth promoter use in livestock.

As a consequence, veterinarians, public health officials, microbiologists and regulatory authorities have become increasingly involved in the investigation and interpretation of the epidemiology, risks, and other pertinent considerations of this antimicrobial use pattern. The literature associated with the benefits and risks of antimicrobial growth promoters is vast and has been accumulating constantly for more than half a century. This chapter provides an introduction to this area, highlighting key findings and issues.

History

The 1940s was a fertile time for nutritional and biochemical investigations. The role of many essential dietary factors, including many vitamins, was discovered. During this decade, increased growth rates in chickens consuming diets supplemented with arsenicals, sulfonamides, streptomycin or chlortetracycline were also observed. However, the era of the antimicro-

bial growth promoters began with an announcement at the American Chemical Society meeting in Philadelphia on 9 April 1950 by Stokstad and Jukes, both pioneers in vitamin research. They described their observations that the addition of the crude mycelial mass produced by the fermentation of *Streptomyces aureofaciens* to the feed of poultry and pigs resulted in spectacular increases in rates of growth. Rather than a simple response to the supply of vitamin B₁₂, as they had initially hypothesised, much of the improved performance was directly attributable to the presence of low concentrations of chlortetracycline.

This serendipitous discovery of antimicrobial growth promotion coincided with a revolution in animal husbandry as pasture production was being replaced by more intensive housing. Much was still to be learned about the nutritional requirements and disease control interventions needed under these new environmental conditions. The advent of antimicrobial growth promotion, however, permitted improved food production at a time of fundamental change and increasing demand.

While much of the initial study of antimicrobial growth promotion concentrated on the tetracyclines and penicillin, other agents were progressively discovered and developed, in many cases displacing their predecessors. Table 24.1 provides an insight into the age of many of the agents still in use in some countries. Notably, there are few examples of antimicrobial growth promoters that have been described in the last 30 years.

Among the numerous observations made in the early decades of antimicrobial growth promoter research, it was noted that unsupplemented control groups of animals had improved weight gains and reduced mortality when raised in the vicinity of groups

Table 24.1. Timeline of milestones in the discovery of antimicrobial drugs with growth promoting activity.

Decade	Compound	Discovery	Other Events
1940s	Penicillin	1940	1940 Florey & Chaim isolate penicillin
	Roxarsone	1941	
	Bacitracin	1945	
	Chlortetracycline	1948	1946 Moore & others*
	Oxytetracycline	1950	1949-1950 Stokstad & others*
1950s	Lasalocid	1951	
	Kitasamycin	1953	
	Virginiamycin	1955	
	Oleandomycin	1956	1959 Transferable resistance first described
	Avilamycin	1961	
1960s	Tylosin	1961	
	Lincomycin	1963	1962 Netherthorpe report*
	Carbadox	1964	1963 <i>Salmonella typhimurium</i> PT29 in UK
	Bambermycins	1965	
	Monensin	1967	
	Avoparcin	1967	
	Olaquinox	1970	1969 Swann report*
			1970 FDA Task Force
1970s	Salinomycin	1972	
	Tiamulin	1973	
	Laidlomycin	1974	
	Narasin	1975	
	Efrotomycin	1975	
1980s	Alexomycin	1989	1980 NAS Study
			1988 IOM Review
1990s	LL-E19020	1989	1988 human VRE infection described
			1997 WHO Consultation
			1998 NRC Report
			1999 GAO Reports
2000s			2000 WHO principles of resistance containment

***Milestones:**

1946. Moore and colleagues first described growth responses to antibiotics.

1949. Stokstad and others announced growth responses to chlortetracycline fermentation mash (mycelium), findings soon to be published on the front pages of the global press.

1962. Lord Netherthorpe chairs committee evaluating whether feeding antibiotics to farm animals constitutes any danger to human health. Found no danger and recommended extension of use to calves.

1969. Professor Swann chairs committee formed to assess likelihood and impact of transferable resistance on human health. Finds risks and significant benefits and presents criteria for selection of feed antibiotics.

receiving antimicrobial growth promoters. This finding was attributed to a reduction in the total environmental load of pathogenic bacteria. Other observations during this period included the retention of the effectiveness of antimicrobial growth promotion after prolonged use (even after decades of use), greater responses in young animals, significant reductions in enteric diseases in supplemented animals, reduction in vitamin and protein requirements, and (not unexpectedly) reduced responses as animals approached their genetic potential for growth. A summary of the diverse

array of physiological, metabolic, nutritional, and disease control effects that have been documented is presented in Table 24.2.

In a statement that remains valid today, having completed a comprehensive review of antimicrobial growth promotion in pigs and poultry, Hays (1979) concluded that

the magnitude of the response to antibacterial agents varies with stage of life cycle, stage of production, and the environmental conditions to

Table 24.2. Some physiological, nutritional and metabolic effects ascribed to antibiotic feed additives.

Effect	Change	Effect	Change
Adverse bacteria	↓	Gut urease	↓
Alpha-toxin production	↓	Gut wall diameter	↓
Ammonia production	↓	Gut wall length	↓
Beneficial bacteria	↑	Gut wall weight	↓
Beneficial <i>E. coli</i>	↑	Limiting amino acid supply	↑
Beneficial lactobacilli	↑	Liver protein synthesis	↑
Calcium absorption	↑	Methane emission	↓
<i>Clostridium perfringens</i>	↓	Mucosal cell turnover	↓
Competition for nutrients by gut flora	↓	Nitrogen excretion	↓
Debilitation of pathogens	↑	Nitrogen retention	↑
Energy retention	↑	Nutrient synthesis by gut flora	↑
Fecal fat excretion	↓	Pathogenic <i>E. coli</i>	↓
Fecal moisture	↓	Pathogenic streptococci	↓
Fatty acid absorption	↑	Phosphorus excretion	↓
Fatty acid oxidation	↓	Plasma nutrients	↑
Feed intake	↔	Stress	↓
Glucose absorption	↑	Toxic amine production	↓
Gut absorptive capacity	↑	Trace element absorption	↑
Gut alkaline phosphatase	↑	Transferable resistance	↔
Gut energy loss	↓	Vitamin absorption	↑
Gut food transit time	↑	Vitamin synthesis	↑

Source: Adapted from Rosen (1995).

which animals are exposed. The response is greater in young animals than in more mature animals. The response is greater during critical stages of production such as weaning, breeding, farrowing or immediately post hatching in chicks and turkeys. Environmental stresses such as inadequate nutrition, crowding, moving and mixing of animals, poor sanitation and high or low temperatures also contribute to increased responses. Such stresses are ordinary and to a large degree unavoidable.

Mechanism of Action

It was recognised very early in the history of antimicrobial growth promotion that the action of antimicrobial agents in increasing growth, feed efficacy and animal health was largely confined to effects on the bacteria within the gastrointestinal tract. This contention rests primarily on the following observations: (1) Antibiotics of widely varying chemical structure are effective, precluding the possibility of incorporation into any growth factor essential for the animal. (2) Antibiotics do not promote growth in germ-free

animals. (3) Antibiotics are ineffective in increasing growth in the developing chick embryo. (4) Sanitation affects the magnitude of the antibiotic growth response. (5) The growth promoting effect is observed with orally administered unabsorbed agents such as bacitracin. And finally, (6) the growth promoting effects of certain parenteral antimicrobials may be explained by their excretion into the intestine.

Many hypotheses have been proposed to explain the mode of action of antimicrobial growth promoters. There remains no unifying principle or single mode of action, and it is likely that different mechanisms predominate in different situations. The magnitude and characteristics of bacterial metabolism in the intestine are dependent on the animal species, age of the host, diet, and portion of the intestinal tract investigated. Interactions between the enteric flora and the host have been described as either competitive or cooperative. Competitive interactions are typical of carnivores, in whom physiological mechanisms (such as low gastric pH and rapid gut transit) have evolved to limit the interaction of flora and nutrients. By contrast, cooperative interactions have evolved in herbivores, notably ruminants, where the host provides optimal conditions for bacterial fermentation. The mode

of action of antimicrobial growth promoters must be consistent with these varying situations.

Among the hypotheses already proposed and tested in monogastric species (poultry, pigs and calves) are the following:

1. Stimulation of intestinal synthesis of vitamins by bacteria. The addition of vitamins at high levels to the diet reduces the response to antimicrobial agents. It has been reported that oral chlortetracycline may increase vitamin availability by increasing fecal elimination of vitamin B₁₂, and that streptomycin has been observed to increase the population of vitamin B₁₂-producing *Bacillus megaterium*.
2. Reduction in total numbers of bacteria in the intestinal tract with a lowering of competition between microorganisms and host animal for nutrients.
3. Inhibition of harmful bacteria which may be mildly pathogenic or toxin-producing. A number of antimicrobial agents have been shown to prevent the growth of *Clostridium perfringens* in the intestinal tract of broilers, turkeys, and pigs. Other researchers have suggested or demonstrated that growth depression is associated with the presence of *Enterococcus faecalis* or *Enterococcus faecium*. Tsinas et al. (1998) observed that in pigs the ability to control *Lawsonia intracellularis* was directly related to growth enhancement. Animals raised in pristine environments benefit less from antibiotic supplementation, while those growing in well established facilities respond sometimes dramatically to the inclusion of antimicrobial agents in their diet, consistent with the presence of growth depressing agents. Bacterial deamination and decarboxylation of amino acids can lead to the production of toxic degradation products. For example, decarboxylation of lysine yields cadaverine, whereas tyrosine and tryptophan are converted to a number of volatile phenolic and aromatic metabolites (including 4-methylphenol and 3-methylindole or skatole), which are both malodorous and potentially toxic. Various antimicrobial growth promoters have been shown to variously decrease the production of these metabolites.
4. Inhibition of bacterial urease. It has been suggested that ammonia produced by bacterial urease damages the intestinal mucosa, impairing nutrient absorption and impeding growth. However, caprylohydroxamic acid, a synthetic urease inhibitor, has been shown to have no effect on growth rate and feed efficiency in chicks.
5. Improved energy efficiency of the gut. The gut attracts a high proportion of cardiac output and contributes a commensurate rate of heat production, parameters that are influenced by nutritional status. Antimicrobial administration has been shown to improve nutrient digestibility and to enhance energy utilization mediated by intestinal microbes. The gut mucosa is the most metabolically active tissue in the body, and it has been demonstrated that antimicrobial supplementation reduces cell turnover in the small intestine and increases the rate of glucose uptake by isolated brush border vesicles.
6. Inhibition of bacterial cholytaurine hydrolase activity. Conjugated bile acids are secreted via the bile into the small intestine where they aid digestion, emulsification and absorption of fats, lipids and fat soluble compounds such as α -tocopherol. Bacteria, principally Gram-positive genera, hydrolyse conjugated bile acids, reducing their function and also increasing the concentration of the hydrolysis product lithocholic acid, which is hepatotoxic and causes inflammation of the small intestine. Feighner and Dashkevich (1987) found an inverse relationship between the growth performance of antibiotics and cholytaurine hydrolase activity, raising the possibility of a discrete mode of action. This hypothesis is supported by recent studies in broilers that have shown high levels of bile salt hydrolase activity expressed by *C. perfringens*. Enzyme activity, unconjugated bile acids, and *C. perfringens* numbers are reduced and ileal absorption of fatty acids was improved by supplementation with avilamycin and salinomycin (Knarreborg et al., 2004).
7. Nutrient sparing. Studies in the early 1950s found that efficient utilization of protein by pigs was obtained only when the diet contained the mycelial Lederle APF (animal protein factor) supplement and observed that a diet containing APF and 18% protein led to equivalent growth rates to pigs consuming a diet with 19.6% protein. It was suggested that the accepted values for the protein requirements of pigs may need to be re-evaluated by

using adequate amounts of vitamin B₁₂ plus other factors present in the Lederle APF supplement in the ration. Many subsequent studies have corroborated this early observation on protein sparing and established that energy, vitamins and minerals can also be spared, with particular significance for reduced inputs and outputs of environmentally important greenhouse gases and nutrients such as nitrogen and phosphorus.

8. Improved nutrient absorption from morphological changes to small intestinal epithelium. A notable feature of germ-free animals and those whose diets are supplemented with antimicrobial growth promoters is a reduction in mass, manifested as shortening and thinning of the intestinal wall. It has been suggested that these changes may allow improved nutrient absorption.
9. Modification of intestinal enzyme activity. The characteristics of intestinal enzyme activity are significantly influenced by the presence of the microflora and factors modifying this ecosystem such as the antimicrobial growth promoters could favourably influence enzyme activity and the availability of nutrients.
10. Reduced immune stimulation. Microbial challenges, while infrequently resulting in clinical disease, do provoke immune responses that are metabolically expensive and lead to increased basal metabolic rate, changes in nutrient absorption, and partitioning of dietary nutrients away from skeletal muscle accretion. It has been demonstrated that dietary antimicrobial supplementation results in improved performance coupled with a reduction in several indicators of immune system activation.

Although it was thought in the 1950s that oral antibiotic administration was detrimental to ruminants, when dose rates were lowered and when novel agents such as the ionophores were introduced in the 1970s significant benefits in performance were realized. An additional mode of action specific to ruminants includes:

11. Modification of rumen microbial metabolism. Fermentative digestion is advantageous for substrates that cannot be digested by host enzymes. However, fermentation results in losses of energy and protein and is therefore disadvantageous for nutrients such as protein, amino acids and sugars readily digested by host enzymes. Optimal pro-

ductivity in ruminants depends on an appropriate balance of fermentative and host digestion. The principal mode of action of most antimicrobial growth promoters in ruminants is to manipulate the ruminal ecosystem. Energetic efficiency is improved by manipulating carbohydrate fermentation in favour of propionate with simultaneous decreased methane production and loss. In addition, starch utilization is improved if the microbiota are shifted away from net lactic acid production. Nitrogen metabolism can be enhanced by reducing bacterial proteolysis and increasing ammonia assimilation. Ruminal lipid metabolism can be favourably manipulated if lipolysis is inhibited, allowing reduced biohydrogenation and increased flow of unsaturated fatty acids to the small intestine.

Advanced molecular biology techniques have allowed fundamental improvements in the understanding of the complex microbial ecology of the gut (Backhed et al., 2005). Bacterial and archaeal genera and species have been studied using specific 16S rRNA-targeted oligonucleotide hybridization probes and denaturing-gradient gel electrophoresis. Such studies have allowed researchers to identify and enumerate the flora of cattle (Stahl et al., 1988), sheep (Edwards et al., 2005), pigs (Collier et al., 2003) and poultry (Knarreborg et al., 2002), and to assess the consequences of challenge with antimicrobial growth promoters, permitting further elucidation of their modes of action.

Regulatory Review Criteria

In common with many other veterinary medicines, the use of antibiotics to improve food animal productivity has been highly regulated over the years. Thorough demonstration of manufacturing quality, efficacy, and safety (including tissue residues, toxicology, target animal safety, occupational safety and environmental safety) are required. Manufacturers of feed additives supply to regulatory agencies environmental toxicology and fate studies that describe the soil half-life of the antibiotic and related metabolites, as well as data on effects on soil-associated organisms, fish, wildlife, and plants. Specific US approval guidance is available at the FDA Center for Veterinary Medicine

(<http://www.fda.gov/cvm/Guidance/published.htm>). Similar requirements apply in other countries, facilitated by the development and adoption of common guidelines by Japan, the US, and Europe under the auspices of the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Products (VICH).

In the US, medicated feed products are classified as Category I or II, and A, B or C (irrespective of the intention of use), depending on the withdrawal time, concentration, and mixing status (Feed Additive Compendium, 2005). A Type A premix contains the highest drug concentration and can be manufactured only under FDA approval and in compliance with Current Good Manufacturing Practices (cGMP). A Type B premix contains a lower concentration of drug than a Type A premix, and can be further mixed. A Type C premix is the final product for feeding that cannot be further mixed. Mixing of category II, Type A premix into a Type B or C feed is done only by FDA-licensed feed mills, which requires establishment registration, full cGMP, and mandatory two-year inspections as conditions for a Feed Mill License. All mixed feeds must display specific labeling information clearly listing ingredients, feeding instructions, cautions or warnings, feed withdrawal information (for unsafe residue avoidance), and other relevant information. The final medicated feedstuff is manufactured at the feedmill to conform to tight inclusion range specifications for potency, then bagged or delivered in bulk to the farm where it is to be used.

The concentration in feed of most antibiotics for growth promotion is in the order of 5–125 ppm (or mg/kg of feed) which equates to only a few mg/kg for the individual animal on a daily intake basis. For example, every kg of a product containing 10 ppm of an antibiotic feed additive contains 10 mg of the antibiotic. If the animal consuming this feed weighs 100 kg, then the intake of antibiotic is 0.1 mg antibiotic per kg bodyweight for every kg of the feed consumed. Analytical assays have been developed for all drug ingredients in order to be able to confirm proper mixing, prevention of cross-contamination, and for other quality-related purposes.

Antibiotics approved as feed additives for growth promotion in the United States are listed in Table 24.3. Products formerly used in the European Union for productivity enhancement are listed in Table 24.4. It is important to note that therapeutic uses for antibiotics

Table 24.3. Antibacterial feed additives approved for growth promotion in cattle, swine and poultry in the United States.

Drug	Antibiotic Class	Cattle	Swine	Poultry
Arsenicals	Arsenical		+	+
Bacitracin	Polypeptide		+	+
Bambermycins	Glycophospholipid		+	+
Carbadox	Quinoxaline		+	
Tetracyclines	Tetracycline	+	+	+
Chlortetracycline, sulfamethazine, penicillin	Combination	+	(<75 lbs)	
Lasalocid	Ionophore	+		
Lincomycin	Lincosamide	+	(>75 lbs)	+
Monensin	Ionophore	+		
Penicillin	Beta-lactam		+	+
Tiamulin	Pleuromutilin		+	
Tylosin	Macrolide	+	+	
Virginiamycin	Streptogramin	+	+	+

Source: Feed Additive Compendium, 2005.

such as tylosin and the ionophores (as anticoccidial agents) remain unaffected by recent EU directives. In 1996, avoparcin was suspended from the list of European Union approved products pending a re-evaluation of the potential medical impact associated with the selection of glycopeptide resistant enterococci by its use. Following this precedent, in late 1998, the European Agriculture Council and Commission voted to invoke the “precautionary principle” for drugs in classes also used in human medicine, namely bacitracin, spiramycin, tylosin and virginiamycin, thereby removing their claims to improve productivity, effective July 1999, resulting in discontinuation of the use of these products for growth promotion. The remaining antibiotics, avilamycin, flavomycin, monensin and salinomycin, although not used in human medicine, have had their productivity claims removed effective January 2006. Other countries use the same products for performance responses. Some countries, such as Japan, also have unique products such as bicozamycin, destomycin, polynactin, sedecamycin, halofuginone, ethopabate, thiopeptin, nosiheptide, enramycin and kitasamycin.

Usage Practices and Benefits

Over the past five decades, numerous changes in the production of food animals have taken place, most no-

Table 24.4. Antibacterial feed additives formerly approved for growth promotion in cattle, swine, and poultry in the European Union.

Drug	Antibiotic Class	Cattle	Swine	Poultry
Avoparcin ¹	Glycopeptide	+	+	+
Bacitracin ²	Polypeptide	+ (calves)	+	+
Flavomycin ³	Glycophospholipid	+	+	+
Monensin ³	Ionophore	+		
Salinomycin ³	Ionophore		+	
Spiramycin ²	Macrolide	+ (calves)	+	+
Tylosin ²	Macrolide		+	
Virginiamycin ²	Streptogramin	+ (calves)	+	+
Avilamycin ³	Orthosomycin		+	+
Carbadox ⁴	Quinoxaline		+	
			(<4 months)	
Olaquinox ⁴	Quinoxaline		+	
			(<4 months)	

¹Suspended, then withdrawn in 1998.²Authorization withdrawn under EU Council Regulation (EC) 2821/98, effective July 1999.³Authorization withdrawn effective January 2006.⁴Authorization withdrawn under EU Commission Regulation (EC) No 2788/98, effective January 1999.

Sources: Lawrence, 1998; Corpet, 1996; Swedish Ministry of Agriculture, 1997.

tably the consolidation of production toward large, company-operated farms which raise the vast majority of livestock and poultry in groups indoors, or outdoors in the case of beef cattle feedlot enterprises. Improvements in animal genetics, herd/flock management, medicinal usage practices, feedstuffs, biosecurity, and hygiene have allowed increased production of meat and other foods of animal origin in a safe, cost-efficient manner to meet the ever-increasing consumer demands for animal protein. The use of antibiotics to facilitate growth promotion has evolved during this period, so that today a wide variety of application strategies have been developed targeting product choice, age of animal medicated, duration of medication, and utilization of professional consultation (Dewey, 1997).

It is generally perceived that the only benefit to the use of antibiotic growth promoters is a positive economic return to the producer, while risks to human health and the environment are ignored. However, the application of antibiotic growth promoters to modern food production programs actually offers a number of significant benefits. It should be noted that not all of the benefits summarized below have been approved by all regulatory authorities as label claims for the antibi-

otics listed, and not all antibiotics that are used in the field are discussed.

There are myriad benefits associated with the use of antibiotic growth promoters; six of the key benefits are summarized below and other benefits are set out in Table 24.5. First, enhancing the efficiency of nutrient utilization by animals allows increased lean muscle gain to be added per pound of feed consumed, resulting in an overall reduction in feed consumption. Logically, reduced feed intake means less cropland, water, and energy are needed for feed production. Second, less feed intake results in reduced fecal output, lessening the environmental burden from excess nutrients such as nitrogen and phosphorus. Third, maintaining a stable fermentation process within the rumen, small intestine, and hindgut of ruminants not only decreases the likelihood of metabolic disorders such as ketosis, but can reduce emissions of methane, an important greenhouse gas. Fourth, by reducing or shifting the populations of certain bacteria in the gut, there is a reduced need for the animal's immune system to respond, thus contributing to a healthier animal and improvement in animal welfare. Fifth, suppression of potential pathogens that may be present in low numbers can prevent important enteric diseases, which in a group setting, benefits overall flock or herd health and welfare. Sixth, recent information suggests that growth promoters reduce the variation in size of slaughter animals and might thereby simplify carcass processing and improve the quality of the meat product.

Performance Benefits

The percentage improvement in performance of pigs fed antibiotics is summarized in Table 24.6. Daily gain refers to the amount of weight added per day, and feed efficiency is a measure of the amount of body weight gain per pound of feed consumed. The efficacy of growth promotion was constant over the two periods compared, and for the two categories of pigs reported. The growth response (measured as percent gain) is greater in the starter than the grower-finisher; an observation consistent with actual use practices (Zimmerman, 1986).

A comprehensive review of published performance responses for the major food animal species during the 1980s was compiled for the Centre for European Agricultural Studies (CEAS, 1991). A total of 235 comparisons of improved growth rates ranged from 4 to 19% and a total of 185 comparisons of feed conversion

Table 24.5. Summary of benefits of antimicrobial growth promoters.

Benefit	Avilamycin	Bacitracin	Bambermycin	Lasalocid	Monensin	Narasin	Salinomycin	Kitasamycin	Oleandomycin	Tylosin	Virginiamycin
Environmental Benefits											
Reduced methane emission (primarily ruminants)			+	+	+	+	+			+	+
Reduced nitrogen excretion (all species)	+	+	+	+	+	+	+			+	+
Reduced phosphorus output (all species)				+	+	+	+				+
Performance Improvements											
Increased rate of bodyweight gain	+	+	+	+	+	+	+	+	+	+	+
Lower feed requirements for each unit of gain	+	+	+	+	+	+	+	+	+	+	+
Improved carcass yield	+		+								
Improved sow performance							+				+
Improved piglet survival & growth							+				+
Increased dairy cow milk production				+	+						+
Increased wool growth			+								+
Disease Control											
Necrotic enteritis in poultry	+	+		+	+	+	+	+			+
Clostridial enteritis in pigs							+				+
Porcine proliferative enteropathy	+	+			+		+			+	+
Swine dysentery							+				+
Acute pneumonia in cattle				+	+	+	+				
Coccidiosis in calves and sheep				+	+	+	+				
Toxoplasmosis in ewes					+						
Prevention Of Metabolic And Fermentative Disorders											
Decreased lactic acidosis				+	+	+	+				+
Decreased laminitis				+	+	+	+				+
Decreased ketosis					+						
Decreased ruminal bloat				+	+						
Other Benefits											
Protein sparing	+	+	+	+	+	+	+			+	+
Energy sparing	+	+	+	+	+	+	+			+	+
Improved mineral absorption				+	+						+
Improved heat tolerance	+			+	+	+	+				+
Decreased boar taint		+									+
Reduction in antibiotic resistance and its transfer		+	+								
Improved immune status		+								+	
Drier litter and reduced foot problems in broilers	+										+
Decreased fly survival in cattle feces				+	+						

Source: Page, 2003.

efficiency yielded improvements in the range 5 to 15% for animals fed antibiotic growth promoters as compared to those that were not. Additional summary data on performance responses can be found in the publications of the Swedish Ministry of Agriculture (1997) and Page (2003).

The economic benefits of using antibiotics in production animals have been described from various perspectives in the United States and Europe, including the consequences of their discontinued use (Zim-

merman, 1986; Swedish Ministry of Agriculture, 1997; Lawrence, 1998; US General Accounting Office, 2004). Whereas the financial return per animal (in terms of gain accrued from the use of a growth promoter) is small, the cumulative effect on an industry that produces millions of cattle, sheep, and pigs and billions of chickens each year is economically significant. For an individual producer, the profit margin attributed to the use of a growth promoter can make the difference between profit and loss.

Table 24.6. Percent improvement in performance of pigs fed antimicrobials for specific periods.

Year	Periods ^a	Improvement (%)	
		Daily gain	Feed/gain
1950 to 1977	Starter	16.1	6.9
	Grower-Finisher	4.0	2.1
1978 to 1985	Starter	15.0	6.5
	Grower-Finisher	3.6	2.4

^aStarter body weight 8–26 kg, grower-finisher 27–92 kg.

Source: Zimmerman, 1986.

Table 24.7. Summary of European performance responses.

Livestock Enterprise	Growth Rate Responses		Feed Conversion Efficiency	
	Number of comparisons	Improvement over control	Number of comparisons	Improvement over control
Broilers	40	+4%	32	+5%
Piglets	30	+19%	28	+15%
Growing pigs	51	+11%	36	+7%
Veal calves	22	+18%	16	+8%
Beef cattle	92	+12%	73	+10%

Source: CEAS, 1991.

A US General Accounting Office report (2004) summarizes several studies that assess the economic effects from discontinuation of antibiotic growth promotion in major food animal species. Several of the studies projected that to maintain animal production in the absence of growth promoters, an increase in the total number animals to produce the same amount of meat would be required and would actually increase the need for environmental resources. In general, the various reports describe a loss to the producer, with minimal food price increases of products at the retail counter.

Environmental Benefits

The environmental benefits of using antibiotics in production animals arise from more efficient production by reducing the time needed to reach market weight, thereby lowering the quantity of feed and water required, hence less nitrogen and phosphorous excretion via urine and feces (Lawrence, 1998; Page, 2003). This improved feed efficiency means less land

(and associated herbicides, fertilizers, agricultural equipment, etc.) required for crop production, as well as reductions in transportation costs for feed, etc. A major benefit for cattle raised with an ionophore such as monensin results from the reduction in the production and emission of methane, an important greenhouse gas (Tedeschi et al., 2003). In four European countries, an annual reduction of approximately 140–190 million cubic meters of methane from cattle was ascribed to the use of monensin (CEAS, 1991).

Prevention of Metabolic and Fermentative Disorders

In cattle, the use of ionophores in particular reduces ketosis and bloat, while virginiamycin reduces the risk of lactic acidosis in sheep and cattle (Page, 2003).

Disease Prevention

The intended use of antibiotics for performance, and not disease prevention, does in fact, suppress subclinical disease associated with bacterial, or in some cases, protozoal pathogens. The rationale is that food animals may be exposed to low numbers of pathogens that occasionally colonize the gut. In spite of the low antibiotic concentrations present, there is sufficient activity to inhibit the small number of susceptible bacteria before they can multiply to achieve a “quorum” that results in clinical disease. Diseases such as necrotic enteritis in poultry, ileitis or clostridial enteritis in swine, and liver abscesses and coccidiosis in cattle may be prevented (Tsinas, 1998; Page, 2003).

Other Benefits

A diversity of other beneficial effects specific to individual antibiotics include improved heat tolerance, increased mineral absorption, and enhanced immune status (Page, 2003).

Public Health Issues of Antibiotic Resistance

The critical public health issue is that of antimicrobial resistance selection by the use of antimicrobial growth promoters in food animals and the potential for resistant food-borne bacteria, or their resistance determinants, to cause a food-borne disease in humans which is subsequently less responsive to treatment. This is also discussed in Chapter 3.

A brief chronology of reviews and major public health actions specific to antibiotics in animal feeds,

mainly in the US, is detailed in Table 24.1 (US Congress OTA, 1995; US FDA, 2000). In the UK, two separate reports of the Netherthorpe Committee (1962, 1966), which had been formed to specifically “examine the possible consequences of the feeding of antibiotics to farm animals”, reviewed the potential public health impacts arising from the use of antibiotics for growth promotion and concluded there was no “reason to discontinue the permitted usage of feed additives”, and indeed recommended that “the use of feed additives could be extended to young calves” (Swann, 1969). However, because of the emergence of transmissible resistance in the form of *Salmonella typhimurium* phage type 29 in calves in the UK in 1963, the Netherthorpe committee recommended a new committee be formed to specifically review “the phenomenon of infective drug resistance, to consider the implications for animal husbandry and also for human and animal health.”

The Swann Committee was established in 1968 with this objective. The final report of the Swann committee made a number of important recommendations about the use of therapeutic and feed additive antibiotics including a recommendation that non-prescription antibiotics used in feed should be restricted to those that “have little or no application as therapeutic agents in man or animals and will not impair the efficacy of a prescribed therapeutic antibiotic . . . through the development of resistant strains of organisms” (Swann, 1969).

Following the recommendations of the Swann Committee in 1969, most growth promoters in Europe were non-therapeutic antibiotics, ionophores, or synthetic compounds, hence the exclusion of tetracyclines and penicillins as growth promoters. During this period, public health officials were mainly concerned with Gram-negative zoonotic bacteria, especially *Salmonella*, *Campylobacter* and *E. coli*. Those antimicrobial growth promoters with primarily a Gram-positive spectrum of activity were thought to have the potential to provide Gram-negative bacteria with a competitive advantage if the protective Gram-positive flora were reduced (i.e., competitive exclusion barrier disruption).

In the US during the 1970s, as a consequence of the UK actions, the FDA conducted several reviews of antibiotic use in animal feeds. A new requirement in the Code of Federal Regulations (21 CFR 558.15) made it necessary for drug sponsors to conduct salmonella

shedding studies and *E. coli* resistance selection studies of feed additive products. In 1977, the Center for Veterinary Medicine issued a Notice of Opportunity for Hearing for subtherapeutic uses of penicillin and tetracycline. In 1978, a Congressional request to the National Academy of Sciences (NAS) was made and the National Research Council undertook a review of the effects of subtherapeutic uses of antibiotics.

In its 1980 report (NAS 1980), while recognizing the potential lack of therapeutic efficacy associated with treating tetracycline-resistant *Salmonella* cases, the committee reported that the available data neither proved nor disproved human health effects from subtherapeutic uses in livestock. The US House Appropriations Committee funded an FDA study in 1981, which was completed in conjunction with the Seattle-King County Department of Public Health in 1984, that concluded that “isolates from human cases and those from retail poultry had similar antibiotic susceptibility patterns, including prevalence of 29.7% and 32.8%, respectively, for tetracycline resistance, which was found to be plasmid-mediated.” During this year, the Secretary for Health and Human Services was petitioned by the Natural Resources Defense Council for suspension of subtherapeutic uses that posed an “imminent hazard”. This was followed by hearings at the House committee level and by the FDA Commissioner as well as a review of the 1984 FDA study. As a result, in 1985, the Secretary denied the petition and in 1987 the FDA requested that the NAS initiate a quantitative risk assessment of the human health consequences associated with the use of penicillin and the tetracyclines at subtherapeutic concentrations in animal feeds. The task was undertaken by the Institute of Medicine (IOM), which concluded that there was no definitive evidence of adverse effects to human health from subtherapeutic uses of antibiotics in food animals, although they believed such effects could exist (IOM, 1989).

In 1988, Uttley et al. were the first to describe infection with vancomycin-resistant enterococci (VRE) in humans, and so commenced an increased recognition of the importance of emerging Gram-positive bacteria as human pathogens. Enterococci are commensal bacteria, playing a vital, beneficial and usually innocuous role as a minor constituent of the bacterial flora of the large intestine of humans and most animal species. However, under special circumstances, especially in the critically ill or immunocompromised patient, en-

terococci (especially *E. faecium* and *E. faecalis*) can translocate from the intestine to cause bloodstream, urinary tract, and other infections, as well as colonize heart valves and implants. Currently, enterococcal infections account for more than 20% of approximately two million nosocomial infections in humans each year in the US. Whereas VRE were present in less than 1% of cases in 1989, the contemporary prevalence in the US is approximately 25%. Public health concern focused on antimicrobial growth promoters when it was realised in the early 1990s that many of the agents in widespread use are active against enterococci, select resistant strains which can be recovered from meat products, and have counterparts in human antimicrobial therapy.

A World Health Organization (WHO) Consultation was convened in Berlin in 1997 to (1) obtain an international consensus on priority medical problems arising from the use of antimicrobials in livestock production, and (2) recommend to WHO the next steps toward the development of guidelines for control and containment of the emergence of medically relevant antimicrobial resistance in food animals. The final report recommended that "the use of any antimicrobial agent for growth promotion in animals should be terminated if it is used in human therapeutics or known to select for cross-resistance to antimicrobials used in human medicine" (WHO, 1997).

This recommendation was subsequently modified by WHO in the Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food (WHO, 2000). Two recommendations were specifically targeted to antimicrobial growth promoter use. Recommendation 18 stated "Use of antimicrobial growth promoters that belong to classes of antimicrobial agents used (or submitted for approval) in humans and animals should be terminated or rapidly phased out in the absence of risk-based evaluations. The termination or phasing-out should be accomplished preferably by voluntary programmes of food animal producers, but by legislation if necessary." Recommendation 19 stated "Risk-based evaluations of all antimicrobial growth promoters should be continued. Characterization of the risk may include consideration of the present and potential future importance of the drug to human medicine, its selection of resistance, the potential exposure to humans from resistant bacteria from food animals, as well as other appropriate scientific factors".

During the late 1990s, as a consequence of the recommendations of WHO (1997), a number of regional and national reviews of antibiotic resistance and food animal antibiotic use were conducted by expert panels appointed by various authorities (e.g., JETACAR (Aus), 1999; UK MAFF, 1998; European Commission SSC, 1999).

The recommendations of the European Commission Directorate for Consumer Policy and Consumer Health Protection's Scientific Steering Committee (SSC) on Antimicrobial Resistance (1999) stated that:

Regarding the use of antimicrobials as growth promoting agents, the use of agents from classes which are or may be used in human or veterinary medicine (i.e., where there is a risk of selecting for cross-resistance to drugs used to treat bacterial infections) should be phased out as soon as possible and ultimately abolished. Efforts should also be made to replace those antimicrobials promoting growth with no known risk of influencing intestinal bacterial infections by non-antimicrobial alternatives. It is essential that these actions are paralleled by the introduction of changes in animal husbandry practices which will maintain animal health and welfare during the phase-out process. Thus, the phase-out process must be planned and co-ordinated since precipitous actions could have repercussions for animal health. Meanwhile, it should be reiterated to manufacturers and farmers that the continuous feeding of antimicrobial growth promoters to food animals for the purpose of disease prevention is a contravention of EU regulations and represents misuse; more effective enforcement measures should be adopted.

The JETACAR recommendations established criteria similar to those of Swann (1969) that needed to be satisfied by antibiotics used for growth promotion. The criteria included: demonstrable efficacy under local farming conditions, rare use of the antibiotic as systemic therapy in humans and animals and use of an antibiotic not considered critical therapy for human use, and unlikelihood of impairing the efficacy of any other prescribed therapeutic antibiotic(s) through the development of resistant strains. The Australian veterinary medicine regulatory authority was charged with using a risk analysis approach, including a cost-

benefit analysis, for antibiotic growth promoters that did not appear to fulfill these criteria. The prioritized review recommended by the JETACAR report included glycopeptides, streptogramins, and macrolides. Avoparcin was withdrawn from the marketplace worldwide and a risk assessment was not completed; virginiamycin has been reviewed and its continued use with label changes has been recommended; and the macrolide review is still pending.

Denmark hosted a WHO consultation in Foulum that evaluated the Danish experience of removing growth promoters from animal production (WHO 2003) and declared the experiment in banning products successful, in spite of an increase in weaner pig mortality and no clear reduction in the prevalence of resistance in human pathogens. An independent review of the effects of the removal of antibiotic growth promoters in Europe concluded that an increase in food animal disease resulted in increased therapeutic use of antibiotics (Casewell et al., 2003). Phillips et al. (2004) published a critical review of the published literature on risks to human health from antibiotic use in food animals and concluded that "there is little evidence that resistant enterococci from animals are a risk to human health".

In the US, the position of the American Veterinary Medical Association (AVMA 2002) states, "The AVMA concludes that currently there is not enough evidence to justify legislative or regulatory prohibition of classes of use of antimicrobials in livestock feeds, whether for therapeutic use or for improving animal growth and feed conversion. All regulatory or legislative actions should be transparent and based on scientific risk analysis". The US Public Health Action Plan to Combat Antibiotic Resistance (US CDC, 2005) lists as Goal #49 "Evaluate the nature and magnitude of the impact of using various antimicrobial drugs as growth promotants in different species, using current animal husbandry practices. Use this information to assist in risk-benefit assessments of such use."

Echoing the position of WHO, coordinated US government agencies, and the American Veterinary Medical Association (AVMA), the approach recommended by many national regulatory agencies in determining whether the use of an antimicrobial agent can be expected to be safe is that of risk assessment. The theory and application of risk assessment to resistance selection by veterinary use of antimicrobial

drugs and consequent impact on human health is a novel extension of traditional risk assessment methods that has been comprehensively reviewed by Cox (2005). Risk assessment is or should be a scientific and evidence-based process with clear description of all data sources, assumptions, and uncertainties. The ideal risk assessment will be supported by a sensitivity analysis of each assumption, allowing its importance to be evaluated. Key areas for further research should be clearly identified as new hypotheses are generated. The output of the risk assessment will be a description of the likelihood of harm to human health, presented as a range of credible values.

In spite of recommendations for evidence-based decision making and risk assessment, concerns among some groups persist and a Citizen's Petition was filed in the US in 2005 requesting the deletion of the "non-therapeutic" claims of feed additives (<http://www.keepantibioticsworking.org/new/index.cfm>).

The only antimicrobial growth promoter for which risk assessments have been published is the streptogramin virginiamycin (Cox and Popken, 2004; Kelly et al., 2004). The US FDA Center for Veterinary Medicine (2004) posted an online draft risk assessment that examined the likelihood of impaired therapeutic efficacy of quinupristin-dalfopristin (QD) as a result of the ingestion of streptogramin-resistant *E. faecium* (SREF) present on food commodities and arising from the use of virginiamycin in livestock. QD is the sole member of the streptogramin class available for parenteral use in humans and is used to treat vancomycin-resistant *E. faecium* infection.

The most recent focus of regulatory attention has been the development of guidelines for the study of the effects of residues of antimicrobial drugs (irrespective of use pattern) on the microbial ecology of the human colon (US FDA CVM, 2005). Key microbiological endpoints are effects on the colonization barrier provided by normal intestinal flora and changes in the populations of antimicrobial-resistant bacteria. Currently there are no widely accepted and validated models of human intestinal flora, and it is anticipated that there will continue to be considerable research to develop a reliable and relevant test system that gains broad acceptance. In the meantime, estimates of acceptable residue levels can be derived from pharmacological data and knowledge of the concentration of microbiologically active residues in the colon, the sensitivity profiles of critical resident flora, and the calcu-

lation of a no-observable-adverse-effect concentration (NOAEC). Further information is presented in Chapter 25.

Judicious Use of Antimicrobial Growth Promoters

Despite the apparently low risk associated with the use of antimicrobial growth promoters, discussed above, it remains important to institute all measures that can mitigate and contain the selection and dissemination of antimicrobial resistance. The AVMA maintains its position on the use of antibiotics in livestock feeds and encourages practices that minimize the need (AVMA, 2002). The World Veterinary Association (WVA) has prepared a policy on the prudent use of antibiotics (WVA, 2000) that describes ten key principles, most of which are as applicable to antimicrobial growth promoters as to therapeutic antimicrobial use. These principles include recognition that antimicrobial agents are tools and not masks of poor practice; introduction of quality assurance programs; observance of label directions; and record keeping, monitoring, and surveillance.

Complementing the WVA principles is the application of the three Rs of animal welfare to the use of antimicrobials—refinement, reduction and replacement. Use of antimicrobial growth promoters can be reduced by only using them when there is evidence that they will provide a benefit. Use can be refined by using them in ways that reduce resistance. For example, Delsol et al. (2005) in a study of pigs found that isolates of enteric bacteria resistant to avilamycin were not detected beyond one week after treatment was withdrawn. This suggests that a withholding period of appropriate duration may minimize resistance dissemination. Replacement of antimicrobial growth promoters is an area of intense research and development effort, and many possible alternatives are becoming available. A recent survey of antimicrobial production in the US suggests that the three Rs are being implemented: The proportion of antimicrobial agents used for growth promotion has fallen from 17% in 2001 to 5% in 2004 (Anonymous, 2005).

The principles of judicious use should continue to be observed and implemented. Routine use without likelihood of benefit is not justifiable. Each decision on use needs to carefully determine the balance of risks and benefits, and to further enhance the benefits

and reduce the risks by astute integration of available options for effective maintenance of health, welfare and production.

Alternatives to Antimicrobial Growth Promoters

Because of concerns regarding the potential for selection of antibiotic-resistant bacteria, residues, and environmental effects attributed to the use of antimicrobial growth promoters, a host of non-antibiotic alternatives are available or are under investigation. In light of the evidence base and quality standards that apply to antibiotic growth promoters, it is important that alternative products meet equivalent standards of efficacy, safety, and quality of manufacture. Sound decisions on product selection can only be made with confidence if the quality and strength of the evidence in support of claims are available for review and regulatory approval.

Although not necessarily representative of the safety and quality of alternatives currently in widespread use, caution and the need for appropriate regulation has been underlined by a number of recent studies. For example, Alcid et al. (1994) recovered an isolate of *E. faecium* bearing the *vanB* gene for vancomycin resistance from a probiotic preparation. Wagner and Cerniglia (2005), in a study of antimicrobial drug susceptibilities of anaerobes from a commercially available competitive exclusion product, found resistant *E. coli*, *Bacteroides* spp. and vancomycin-resistant *Lactococcus lactis*, and Ward et al. (2002) found that a variety of herbal products which included garlic and Echinacea caused large increases in the MIC of ampicillin in *E. coli* and *Staphylococcus aureus*.

Resistance to heavy metals is widespread in the environment and resistance genes are commonly situated on plasmids. Two heavy metals, zinc and copper, are widely used for growth promotion. Hasman and Aarestrup (2005) could not exclude the possibility that the use of copper in the diets of pigs in Denmark delayed the elimination of glycopeptide resistance *E. faecium*. Finally, Weese (2002), who investigated the composition of a variety of probiotic preparations, had to conclude that most preparations studied were not accurately represented by their label claims.

A comprehensive summary of many of the currently available alternatives is beyond the scope of this chapter, although a more thorough discussion can be found

elsewhere (Page, 2003). A brief summary of products used in the feed or via systemic administration are described. In addition, improvements in animal husbandry, genetics and nutrition also have a profound and positive impact on animal production and health.

Probiotics and competitive exclusion products (direct-fed microbials) (e.g., *Saccharomyces cerevisiae*, *Lactobacillus*, and other microorganisms) are included in feeds as live microbial supplements. There are multiple modes of action which include competing against pathogens in the gut for binding sites or nutrients, stimulating the gut immune system, and production of bacteriocins such as nisin and lactocidin. Variable improvements in growth responses have been observed.

Prebiotics are indigestible carbohydrates that stimulate "beneficial" intestinal microflora. The best known examples include the oligosaccharides such as mannanoligosaccharide. Trends toward performance improvements have been observed.

Enzymes such as phytase release phosphorus from orthophosphate groups, improving phosphorus bioavailability and reducing excretion. Other enzymes such as xylanase and glucanase break down plant-based feeds, allowing access to energy from complex carbohydrates. Variable responses in weanling pigs have been reported. Herbal additives such as essential oils, spices, and other plants have not generated a consistent disease prevention or performance response in swine studies.

Organic acids such as propionic, formic, fumaric, citric, and lactic acid can, as acidifiers, be inhibitory to enteric bacteria and improve overall performance by reducing competition for nutrients and reducing sub-clinical infections or production of bacterial toxins. The best responses are in young pigs. Immune system stimulators such as spray-dried plasma, egg yolk antibodies and conjugated linoleic acid feed supplementation may provide a degree of protection against pathogens.

β -Adrenergic agonists (such as ractopamine) act as repartitioning agents to modify carcass composition by shifting nutrient partitioning to increase muscle protein content and reduce fat deposition. Ractopamine, a non-hormonal, non-antibiotic agent, increased growth rate (9%) and skeletal muscle (12%), and reduced feed:gain (12%) and adipose tissue (14%) (Page, 2003). Ractopamine has been approved by regulatory authorities in several countries (e.g., US, Australia) but not Europe.

Recombinant bovine and porcine somatotropins (rbST and rpST) have been associated with large increases in animal productivity. Bovine somatotropin results in increased milk yield and an unprecedented improvement in efficiency. Porcine somatotropin results in lean muscle deposition. Until sustained delivery systems are available, both products are injected on a daily basis. These products have been approved for use in several countries.

Anabolic hormonal growth implants, such as estrogens, are used as implants in the ears of cattle. Heavy metals such as zinc, copper or chromium have been shown to decrease the incidence of post-weaning scours in pigs, although there are concerns about antibiotic resistance selection and excretion of the metals into the environment.

Management practice improvements such as providing newborn calves with colostrum, allowing a longer weaning duration for piglets, and management of swine production as an "all in—all out" process have resulted in improved health and performance. Improvements to biosecurity, air quality, and stocking densities are now common and are associated with improved production. Improvements in chicken and swine breeds using genetic selection are ongoing and disease-resistant pigs and dairy cattle are being developed. Nutritional improvement practices, especially precision diet formulation, to achieve optimal diets with an appropriate balance of amino acids, vitamins, minerals, and carbohydrates are continuously being refined.

Bibliography

- Alcid DV, et al. 1994. Probiotics as a source of *Enterococcus faecium*. Orlando, Florida: 32nd Infectious Diseases Society of America Annual Meeting, Abst 123.
- Anonymous. 2005. US antibiotic production rises slightly, as prophylactic use falls. *Animal Pharm* 568(1):16.
- American Veterinary Medical Association (AVMA). 2002. Position on Antimicrobials in Livestock Feeds. http://www.avma.org/issues/policy/jtua_feeds.asp.
- Backhed F, et al. 2005. Host-bacterial mutualism in the human intestine. *Science* 307:1915.
- Casewell M, et al. 2003. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *J Antimicrob Chemother* 52:159.
- Centre for European Agricultural Studies (CEAS). 1991. The impact on animal husbandry in the European Community of the use of growth promoters. Volume 1. Growth

- promoters in animal feed. Report to the European Commission.
- Collier CT, et al. 2003. Molecular ecological analysis of porcine ileal microbiota responses to antimicrobial growth promoters. *J. Anim Sci* 81:3035.
- Corpet DE. 1996. Microbiological hazards for humans of antimicrobial growth promoter use in animal production. *Rev Med Vet* 147:851.
- Cox LA Jr, Popkin DA. 2004. Quantifying human health risks from virginiamycin used in chickens. *Risk Analysis* 24:271.
- Cox LA Jr. 2005. Quantitative health risk analysis methods: modelling the human health impacts of antibiotics used in food animals. In: *International Series in Operations Research and Management Science*, Vol. 82. New York: Springer Verlag.
- Delsol AA, et al. 2005. Effect of the growth promoter avilamycin on emergence and persistence of antimicrobial resistance in enteric bacteria in the pig. *J Appl Microbiol* 98:564.
- Dewey CE, et al. 1997. Associations between off-label feed additives and farm size, veterinary consultant use, and animal age. *Prev Vet Med* 31:133.
- Edwards JE, et al. 2005. Influence of flavomycin on ruminal fermentation and microbial populations in sheep. *Microbiology (Reading)* 151:717.
- European Commission Directorate for Consumer Policy and Consumer Health Protection. 1999. Opinion of the Scientific Steering Committee (SSC) on antimicrobial resistance. http://europa.eu.int/comm/food/fs/sc/ssc/out50_en.pdf.
- Feighner SD, Dashkevich MP. 1987. Subtherapeutic levels of antibiotics in poultry feeds and their effects on weight gain, feed efficiency, and bacterial cholytaurine hydrolase activity. *Appl Environ Microbiol* 53:331.
- Hasman H, Aarestrup FM. 2005. Relationship between copper, glycopeptide, and macrolide resistance among *Enterococcus faecium* strains isolated from pigs in Denmark between 1997 and 2003. *Antimicrob Agents Chemother* 49:454.
- Hays VW. 1979. The Hays Report. Effectiveness of feed additive usage of antibacterial agents in swine and poultry production. Long Beach, CA: Rachelle Laboratories.
- Feed Additive Compendium. 2005. Minnetonka, MN: Miller Publishing Co.
- Institute of Medicine (IOM). 1989. Human health risks with the subtherapeutic use of penicillin or tetracyclines in animal feed. Washington, DC: National Academy Press.
- Joint Expert Technical Advisory Committee on Antibiotic Resistance (JETACAR). 1999. The use of antibiotics in food-producing animals: antibiotic-resistant bacteria in animals and humans. Commonwealth Department of Health and Aged Care and the Commonwealth Department of Agriculture, Fisheries and Forestry, Australia. [http://www.health.gov.au/internet/wems/publishing.nsf/content/health-pubs-jetacar-cnt.htm/\\$FILE/jetacar.pdf](http://www.health.gov.au/internet/wems/publishing.nsf/content/health-pubs-jetacar-cnt.htm/$FILE/jetacar.pdf).
- Kelly L, et al. 2004. Animal growth promoters: To ban or not to ban? A risk assessment approach. *Int J Antimicrob Agents* 24:7.
- Knarreborg A, et al. 2002. Effects of dietary fat source and subtherapeutic levels of antibiotic on the bacterial community in the ileum of broiler chickens at various ages. *Appl Environ Microbiol* 68:5918.
- Knarreborg A, et al. 2004. Dietary antibiotic growth promoters enhance the bioavailability of α -tocopheryl acetate in broilers by altering lipid absorption. *J Nutr* 134:1487-1492.
- Lawrence K. 1998. Growth promoters in swine. *Proc 15th Int Pig Vet Soc Cong*.
- National Academy of Sciences. 1980. The effects on human health of subtherapeutic use of antimicrobials in animal feeds. Washington, DC: National Academy Press.
- Page SW. 2003. The role of enteric antibiotics in livestock production: a review of published literature. Canberra, ACT, Australia: Avicare Limited. <http://www.animal-healthalliance.org.au/files/animalhealth/information/The%20Role%20of%20enteric%20antibiotics%20in%20live-stock%20production.pdf>.
- Phillips I, et al. 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *J Antimicrob Chemother* 53:28.
- Rosen GD. 1995. Antibacterials in poultry and pig nutrition. In: *Biotechnology in Animal Feeds and Animal Feeding*. Wallace RJ and Chesson A, eds. Weinheim, FRG: VCH Verlagsgesellschaft mbH, 143.
- Stahl DA, et al. 1988. Use of phylogenetically based hybridization probes for studies of ruminal microbial ecology. *Appl Environ Microbiol* 54:1079-1084.
- Swann MM. 1969. Joint Committee on the use of antibiotics in animal husbandry and veterinary medicine. London: Her Majesty's Stationery Office.
- Swedish Ministry of Agriculture. 1997. Antimicrobial feed additives. Food and Fisheries SOU 1997:132. Stockholm, Sweden. <http://www.sweden.gov.se/sb/d/574/a/54899>.
- Tedeschi LO, et al. 2003. Potential environmental benefits of ionophores in ruminant diets. *J Environment Qual* 32:1591.
- Tsinas AC, et al. 1998. Control of proliferative enteropathy in growing/fattening pigs using growth promoters. *Journal of Vet Med* 45B: 115.
- UK Ministry of Agriculture, Fisheries, and Food (MAFF). 1998. A review of antimicrobial resistance in the food chain. <http://archive.food.gov.uk/maff/pdf/resist.pdf>.
- US Centers for Disease Control and Prevention (CDC). 2005. A public health action plan to combat antibiotic resistance. <http://www.cdc.gov/drugresistance/actionplan/>.
- US Congressional Office of Technology Assessment. 1995. Impacts of antibiotic-resistant bacteria. OTA-H-629. Washington, DC: US Government Printing Office.
- US FDA Center for Veterinary Medicine. 2000. HHS response to House Report 106-157: Agriculture, Rural Development, Food and Drug Administration, and related agencies, Appropriations Bill. http://www.fda.gov/cvm/HRESP106_157.htm.
- US FDA Center for Veterinary Medicine. 2004. Risk assessment of streptogramin resistance in *Enterococcus faecium* attributable to the use of streptogramins in animals. http://www.fda.gov/cvm/Documents/SREF_RA_FinalDraft.pdf.

- US FDA Center for Veterinary Medicine. 2005. Studies to evaluate the safety of residues of veterinary drugs in human food: general approach to establish a microbiological ADI. Guidance for Industry 159, VICH GL-36. <http://www.fda.gov/cvm/Documents/guide159.pdf>.
- US General Accounting Office (GAO). 2004. Antibiotic resistance: Federal agencies need to better focus efforts to address risk to humans from antibiotic use in animals. GAO-04-490. <http://www.gao.gov/new.items/d04490.pdf>.
- Uttley AH, et al. 1988. Vancomycin-resistant enterococci. *Lancet* i:57.
- Wagner RD, Cerniglia CE. 2005. Antimicrobial susceptibility patterns of competitive exclusion bacteria applied to newly hatched chickens. *Int J Food Microbiol* 109:349-352.
- Ward P, et al. 2002. Inhibition, resistance development, and increased antibiotic and antimicrobial resistance caused by nutraceuticals. *J Food Protect* 65:528.
- Weese JS. 2002. Microbiologic evaluation of commercial probiotics. *J Am Vet Med Assoc* 220:794.
- World Health Organization (WHO). 1997. The medical impact of antimicrobial use in food animals. WHO/EMC/ZOO/97.4. http://whqlibdoc.who.int/hq/1997/WHO EMC_ZOO_97.4.pdf.
- WHO. 2000. WHO global principles for the containment of antimicrobial resistance in animals intended for food. WHO/CDS/CSR/APH/2000.4. http://whqlibdoc.who.int/hq/2000/WHO_CDS_CSR_APH_2000.4.pdf.
- WHO. 2003. Impacts of antimicrobial growth promotion termination in Denmark. WHO/CDS/CPE/ZFK/2003.1. http://whqlibdoc.who.int/hq/2003/WHO_CDS_CPE_ZFK_2003.1.pdf.
- World Veterinary Association (WVA). 2000. WVA Policy on the prudent use of antibiotics. <http://www.worldvet.org/manuals/T-3-2.pdf>.
- Zimmerman DR. 1986. Role of subtherapeutic levels of antimicrobials in pig production. *J Anim Sci* 62 Suppl 3:6.

Antimicrobial Drug Residues in Foods of Animal Origin

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Regulation of Veterinary Drugs

Livestock and poultry production depend on drugs and other chemicals to protect animal health. Food producing animals may be exposed to environmental contaminants or be the targets of bioterrorism. To protect consumers from adverse health effects, federal programs are charged with the regulation of chemicals and drugs and the detection of chemical and drug residues in foods of animal origin. For example, in the United States the US Food and Drug Administration (FDA) and in Canada the Canadian Veterinary Drugs Directorate (VDD) approve veterinary drugs and establish the acceptable concentrations of drug residues in animal-origin food products. The US Department of Agriculture's Food Safety and Inspection Service (FSIS) and the Canadian Food Inspection Agency (CFIA) monitor meat, poultry, eggs and honey for residues of drugs and chemicals. Monitoring of milk and dairy products is mainly carried out on a state or provincial basis. The US and Canadian agencies use hazard analysis and critical control point (HACCP) based systems consistent with the principles of risk analysis. The Codex Alimentarius Committee on Residues of Veterinary Drugs in Foods is a subsidiary body of the World Health Organization and the Food and Agriculture Organization. This Codex committee facilitates world trade in agricultural commodities through the establishment of internationally recognized standards, codes of practice, guidelines, and recommendations that are based on the consensus of expert scientific opinion. A primary function is the establishment of internationally acceptable concentrations of veterinary drugs in food animal products.

Using the US and Canada as an example, before any drug can be approved for use in a food-producing an-

imal in these countries, an extensive toxicologic evaluation of the drug and its metabolites is undertaken. This ensures that any drug residues in animal-derived foods do not harm the consumer. A battery of four toxicologic tests is required to satisfy human food-safety requirements for any new animal drug intended for use in a food-producing animal species:

- (1) Metabolism studies for identification of residues for toxicological testing. This includes metabolite identification in the target species and metabolite identification in a laboratory animal species.
- (2) Toxicological testing in laboratory animals. Genetic toxicity tests, acute toxicity tests, sub-chronic (90-day) toxicity tests, and a two- to three-generation reproduction study with a teratology component in rats. Lifetime carcinogenicity studies in two rodent species *only* if genetic toxicity tests indicate that the drug or metabolites are potentially carcinogenic (the decision by FDA to require lifetime carcinogenicity studies is based on a decision tree process referred to as a *threshold assessment*). Other specific toxicity tests are performed if needed.
- (3) Residue depletion studies in the target species.
- (4) Regulatory analytical methodology for identification and quantitation of marker residues in animal tissues, milk, or eggs. A determinative method for quantitation of marker residues and confirmatory method for structural identification are required when the determinative method is not sufficiently specific.

Drug approval requirements for the US are available from the FDA in guidance documents at <http://www.fda.gov/cvm/Guidance/published.htm>, and in Canada

from the VDD guidelines at http://www.hc-sc.gc.ca/dhp-mps/vet/applic-demande/guide-ld/index_e.html.

Based on the results of toxicity tests, regulatory agencies establish an acceptable daily intake (ADI). The ADI represents a level of daily intake of a chemical which, during an entire lifetime, appears to be without appreciable risk to the health of the consumer. The ADI is used to determine the maximum concentration of a marker residue in edible tissues, honey, milk, or eggs that is legally permitted or recognized as acceptable. In the US, these acceptable concentrations are termed "tolerances" while in Canada and the European Union they are termed "maximum residue limits" (MRLs).

The MRL is calculated such that daily intake of food with residues at the MRL will result in a total daily consumption of residues in quantities at or below the ADI. ADIs are based on the total residue of a chemical present in food (parent compound and all metabolites) whereas MRLs are based on a single, measurable marker residue which may be the parent compound or any of its metabolites. In establishing MRLs, consumption estimates for the various foods are taken into account so that foods consumed infrequently or in small amounts are allowed greater MRL values than those foods likely to be consumed daily or which represent a major component of the diet. Because of differences in consumption factors, MRLs and label withdrawal times may differ between countries, even though ADIs are equivalent (Fitzpatrick et al., 1995, 1996) (Table 25.1). The United States residue limits (tolerances) can be found at http://www.fsis.usda.gov/OPHS/red_book_2001/2001_Residue_Limits_Veterinary_Drugs_App4.pdf, and Canadian MRLs can be found at http://www.hc-sc.gc.ca/dhp-mps/vet/mrl-lmr/mrl-lmr_versus_new-nouveau_e.html.

Causes and Incidence of Residue Violations in the United States and Canada

Drugs, pesticides, environmental contaminants, and naturally occurring toxicants can leave residues in meat, milk, eggs, and honey. Of these, drugs are the most frequently detected chemicals and the overwhelming majority of violations are from antimicrobials. Each year, FSIS analyzes approximately 302,000 samples and the CFIA analyses 220,000 samples from all market classes of food-producing animals. When a

Table 25.1. Estimated consumption of animal products by the extreme (90th) percentile consumer.*

Food	United States (g/day)	Canada (g/day)
Beef Muscle	155	206
Beef Liver	20	20
Swine Muscle	95	98
Chicken Muscle	54	84
Fluid Milk	690	677

*Fitzpatrick et al., 1996.

violative chemical residue is detected in an animal presented for slaughter or in a food animal product, the FSIS or the CFIA condemns the adulterated product. FSIS notifies FDA of residue violations and assists in obtaining the names of producers and, in the case of food animal products, other parties involved in offering the animals or products for sale. The federal agencies take appropriate action when a violation is detected. These actions include follow-up inspections, further directed sampling according to a surveillance plan, or even seizure and recall of products when the human health risk is considered unacceptable. Follow-up actions vary according to the magnitude of the health risk; emphasis is on prevention of repeat occurrence or further distribution of contaminated products.

When approved veterinary drugs are administered according to their label directions, the prevalence of violative drug residues in animal products should be less than 1%. Residue violation rates greater than 1% indicate that a drug has been used in a manner inconsistent with label directions.

From 1960 to 1972, the prevalence of violative antimicrobial drug residues in swine, lambs, calves, and fat cattle slaughtered in the US was 30, 21, 18, and 7%, respectively (Huber, 1971). Prior to 1962, approximately 13% of all milk produced in the US contained residues of antimicrobial drugs (Huber, 1971). Since the 1960s, the prevalence of residues in food animal products has declined significantly but there are still some problems.

Several factors contribute to the drug residue problem, but most violations result from use of veterinary drugs in some manner that is inconsistent with the labeling. Analysis of the probable causes for violative residues in the US reveals that failure to observe withdrawal times, drugs administered in error, treatment

of animals with greater than labeled doses, failure to use the appropriate route of administration, and improper maintenance of medication records are identifiable risk factors (Paige et al., 1999). Medicated feeds are a frequent cause of residue violations in market hogs and poultry. Adherence to medicated feed withdrawal times may be burdensome, inconvenient, and expensive, in that non-medicated feed must be provided during the withdrawal period and this requires the changing of feed programs and containers at the end of the feeding period (Huber, 1971). Lack of treatment records or failure to adequately identify treated animals can lead to insufficient withdrawal periods. When drugs are administered to animals at dosages greater than those specified in the labeling, or when drugs are used in species for which they are not approved, the prescribing veterinarian is responsible for withdrawal recommendations. Recommendations made by veterinarians are often rough estimates and may be inadequate for depletion of drug residues from the carcass, milk, honey, or eggs. Salvaging ill animals for slaughter that have been treated with antimicrobial drugs is a common cause of violative drug residues, especially in culled dairy cows and veal calves. Educational intervention during follow-up investigations by regulatory authorities prevents similar events from recurring in the future.

Federal agencies have residue detection programs for domestically produced products and for imported products. There are four components of the domestic residue sampling plan: monitoring, enforcement, surveillance, and exploratory sampling.

In monitoring programs, a statistically based selection of random samples from healthy animals is collected at slaughter. These samples are analyzed for specific chemical compounds. The number of samples analyzed provides a 95% probability of detecting at least one violation when 1% of the animal population sampled contains residues at violative concentrations. Generally, the samples are from animals that have passed ante- and post-mortem inspections, or are from egg products that have passed inspection. Products are not retained pending laboratory analysis. If results indicate a potential public health concern, however, the products may be recalled. Data obtained from the monitoring program are used to evaluate residue trends. For example, if data indicate a drug is being misused, a targeted sampling program is instituted.

Table 25.2. Antimicrobial residues detected in the United States in 2003 by the FSIS Monitoring Program (5,608 samples tested).

Animal Class	(# of Violations)	Drug	%
Dairy cows	(1)	penicillin	1.0
	(1)	gentamicin	
Bob veal	(16)	neomycin	6.0
	(1)	gentamicin	
Formula fed veal	(4)	neomycin	1.2
Non-formula fed veal	(9)	neomycin	5.6
Heavy calves	(1)	penicillin	0.8
	(1)	gentamicin	
Sows	(1)	penicillin	0.3
Rabbits	(1)	penicillin	2.0

Table 25.3. Sulfonamide residues detected in the United States in 2003 by the FSIS Monitoring Program (5,276 samples tested).

Animal Class	(# of Violations)	Drug	%
Bulls	(1)	sulfadimethoxine	0.3
Steers	(1)	sulfamethazine	0.3
Beef cows	(1)	sulfadimethoxine	0.4
Dairy cows	(2)	sulfadimethoxine	1.4
	(2)	sulfadimethoxine	1.2
Bob veal	(1)	sulfadiazine	
	(2)	sulfamethazine	1.2
Non-formula fed veal	(1)	sulfamethazine	0.4
Heavy calves	(2)	sulfamethazine	0.7
Market hogs	(1)	sulfamethazine	5.6
Roaster pigs	(1)	sulfamethazine	

Residue Monitoring Programs

The results of US and Canadian residue testing programs are available from 2003 (FSIS, 2003; CFIA, 2003). The FSIS analyzed 26,214 monitoring samples in 2003, and found a total of 87 residue violations. The FSIS analyzed 5,608 samples for antimicrobial residues and found 36 violations (Table 25.2) and 5,276 samples for sulfonamides and found 14 violations (Table 25.3).

In the Canadian National Chemical Residues Monitoring Program in 2002/2003, meat and poultry products were randomly sampled from a wide variety of animal classes. Antimicrobial residues were mainly

Table 25.4. Antimicrobial and sulfonamide residues detected in Canada in the CFIA Monitoring Program in 2002/2003.

Animal Class (# of Tests)	(# of Violations)	Drug
Beef (864)	(1)	ciprofloxacin
	(5)	microbial growth inhibitors
Cull cows (943)	(1)	sulfamethazine
Veal	(7)	oxytetracycline
	(1)	penicillin G
Bison (753)	(5)	microbial growth inhibitors
Chicken (1262)	(2)	microbial growth inhibitors
Turkeys	(10)	microbial growth inhibitors
Duck (229)	(2)	microbial growth inhibitors
Game birds (644)	(1)	microbial growth inhibitors
Ostriches (221)	(2)	microbial growth inhibitors
Pork (1293)	(11)	microbial growth inhibitors
	(8)	oxytetracycline
	(1)	sulfamethazine
Sows (1185)	(13)	microbial growth inhibitors
	(2)	sulfamethazine
Horse (1006)	(17)	microbial growth inhibitors
Mutton (1047)	(1)	enrofloxacin
Rabbit (312)	(42)	microbial growth inhibitors
Honey (1055)	(2)	erythromycin
	(10)	tylosin
	(2)	sulfathiazole
	(14)	oxytetracycline
	(10)	tetracycline

screened using microbial inhibition tests. The presence of inhibitory substances, while suggesting antimicrobials, does not prove a legal violation occurred. Fluoroquinolones and chloramphenicol were screened for specifically (Table 25.4).

Residue Enforcement Programs

In contrast to the monitoring programs, which sample healthy animals on a random basis, the enforcement programs analyze samples from animals at high risk of having violative drug residues. In-plant tests used during enforcement testing provide a rapid screening method to detect the presence of residues. Such tests include the Sulfa-On-Site (SOS), Calf Antibiotic and Sulfonamide Test (CAST), Swab Test on Premises (STOP), and the Fast Antimicrobial Screen Test (FAST).

An animal may be suspect because of historical information on a production class or appearance on

ante- and post-mortem inspections. Typical suspect animals include culled dairy cows, bob veal calves (calves <3 weeks of age and weighing <68 kg), any animal with visible evidence of an injection site, animals showing evidence of an infectious disease, or animals of a given production class for which a high incidence of residue violations has been detected through the monitoring program. Carcasses from suspect animals sampled under the enforcement programs are retained at the abattoirs until the results of residue testing are available. When an in-plant screening test detects a positive sample, a confirmation test is conducted at an FSIS or CFIA laboratory. If an in-plant test is not available or if the presence of a chemical residue that cannot be detected by STOP or FAST is suspected, the appropriate tissue samples are sent directly to an FSIS or CFIA laboratory. Carcasses sampled for enforcement testing are retained pending laboratory results. If a violative level is found, the carcass is deemed adulterated and is condemned.

Data from enforcement programs are periodically evaluated to determine whether interventions have been effective in reducing the occurrence of residues. Of the 230,351 enforcement samples analyzed by the FSIS in 2003, 1,923 residue violations were found. The residue violations consisted of 1,470 antimicrobial violations, 118 flunixin violations, and 335 sulfonamide violations. No violations were found in the testing for arsenicals, chloramphenicol, diethylstilbestrol, and zeranol. The FSIS used STOP tests to screen 14,360 animals for antimicrobial, sulfonamide, and non-steroidal anti-inflammatory residues (Table 25.5). Seventy-four FSIS laboratory-confirmed violations were found in 64 animals. The FSIS used FAST to screen 215,813 animals for antimicrobial, sulfonamide, and non-steroidal anti-inflammatory residues (Table 25.6). The FSIS laboratories confirmed 1,820 violations in 1,665 animals. The high rate of neomycin violations in veal calves was mainly due to neomycin-medicated milk replacers fed to calves with enteritis. In normal calves, the oral bioavailability of aminoglycosides such as neomycin is very poor. But with inflammation and damage to the mucosal barriers with enteritis, sufficient quantities of neomycin are absorbed systemically to result in violative kidney residues.

In the Canadian enforcement program in 2002/2003, 11,673 STOP tests and 204 CAST tests were performed on suspect animals. The CFIA laboratories confirmed violative antimicrobial and sulfonamide

Table 25.5. Antimicrobial and sulfonamide residues detected in 2003 in the United States by the FSIS Enforcement Program using STOP testing on 14,360 samples.

Animal Class	(# of Violations)	Drug
Bulls	(1)	penicillin
	(1)	tilmicosin
	(1)	sulfadimethoxine
Steers	(1)	penicillin
Beef cows	(4)	gentamicin
	(1)	oxytetracycline
	(7)	penicillin
Heifers	(1)	sulfadimethoxine
	(1)	neomycin
	(2)	penicillin
Dairy cows	(2)	gentamicin
	(1)	neomycin
	(3)	oxytetracycline
	(28)	penicillin
	(1)	tetracycline
	(3)	tilmicosin
	(4)	sulfadimethoxine
	(5)	flunixin
	(1)	gentamicin
	(1)	oxytetracycline
Lambs	(1)	gentamicin
Goats	(1)	oxytetracycline
Market hogs	(1)	chlortetracycline
	(1)	penicillin
Sows	(3)	penicillin

residues in 346 samples, with oxytetracycline and penicillin G residues in beef and pork being the most common violations (Table 25.7). As in the US, producers and distributors of food who violate Canadian standards are placed on enhanced inspection in order to identify the causes and reduce or prevent recurrences of violations.

Residue Surveillance Programs

Surveillance sampling is scheduled to address residue concerns in a specific animal population. Data collected by surveillance sampling measure the extent of a chemical residue problem in a suspect population or product. Surveillance sampling allows agencies to determine whether or not interventions have reduced the occurrence of residues. Depending on the weight of evidence that led to testing, sampled carcasses or products may be held pending laboratory results. In 2003, 6,295 market hogs were screened by FSIS for sulfonamides using the Sulfa-On-Site test (SOS), with 10 sulfamethazine violations detected. Bob veal surveil-

Table 25.6. Antimicrobial and sulfonamide residues detected in 2003 in the United States by the FSIS Enforcement Program using FAST Testing on 215,813 samples.

Animal Class	(# of Violations)	Drug
Bulls	(1)	gentamicin
	(1)	sulfamethazine
	(1)	flunixin
Steers	(4)	penicillin
	(4)	tilmicosin
	(9)	sulfamethazine
Beef cows	(4)	gentamicin
	(1)	neomycin
	(4)	oxytetracycline
	(30)	penicillin
	(1)	tetracycline
	(3)	tilmicosin
	(8)	sulfamethazine
	(5)	sulfadimethoxine
	(1)	sulfathiazole
	(1)	flunixin
Heifers	(3)	gentamicin
	(1)	neomycin
	(1)	penicillin
	(2)	tilmicosin
	(195)	gentamicin
	(31)	neomycin
	(39)	oxytetracycline
	(552)	penicillin
	(19)	tetracycline
	(24)	tilmicosin
Dairy cows	(3)	tylosin
	(43)	sulfamethazine
	(199)	sulfadimethoxine
	(1)	sulfathiazole
	(111)	flunixin
	(22)	gentamicin
	(372)	neomycin
	(15)	oxytetracycline
	(35)	penicillin
	(2)	tetracycline
Bob veal (including surveillance testing)	(1)	tilmicosin
	(2)	tylosin
	(26)	sulfamethazine
	(3)	sulfamethoxazole
	(13)	sulfadimethoxine
	(1)	gentamicin
	(1)	gentamicin
	(2)	penicillin
	(1)	tilmicosin
	(2)	tylosin
Formula fed veal	(1)	gentamicin
	(1)	gentamicin
	(2)	penicillin
	(1)	tilmicosin
	(3)	gentamicin
	(4)	penicillin
Heavy calves	(2)	tilmicosin
	(2)	sulfamethazine
	(6)	sulfadimethoxine

Table 25.7. Antimicrobial and sulfonamide residues detected in 2002/2003 in Canada by the CFIA Enforcement Program using SOS, STOP and CAST testing on 11,673 samples.

Animal Class	(# of Violations)	Drug
Pork	(41)	oxytetracycline
	(133)	penicillin G
	(2)	tilmicosin
	(17)	sulfamethazine
	(1)	sulfadiazine
	(1)	sulfadimethoxine
	(1)	sulfadoxine
Beef	(7)	ceftiofur
	(78)	oxytetracycline
	(43)	penicillin G
	(3)	tetracycline
	(2)	tilmicosin
	(1)	ciprofloxacin
	(1)	enrofloxacin
	(1)	sulfadiazine
	(4)	sulfamethazine
	(1)	penicillin G
Game bird	(6)	penicillin G
Horse	(2)	tetracycline
	(1)	sulfathiazole
Mutton	(1)	oxytetracycline

lance testing was reported with enforcement testing. A small number of show animals were tested for antimicrobials and sulfonamides, and no violations were found.

Residue Exploratory Programs

Exploratory sampling monitors residues of drugs or chemicals for which MRLs have not been established. It allows agencies to evaluate new methods and approaches for monitoring and sampling and provides information to supplement the monitoring program. Exploratory sampling is for information purposes, and no regulatory action is taken based on the analyzed samples. In 2003, exploratory testing by FSIS was done for heavy metals, but not for antimicrobials or sulfonamides.

Residues in Imported Food Animal Products

Even though animal and egg products imported to the US or Canada have been subjected to inspection in their country of origin, they are reinspected when they enter the US or Canada. The level of reinspection by

the FSIS or CFIA depends on the exporting country's performance history. Import sampling is designed to verify the equivalence of chemical residue programs in countries exporting meat, poultry, honey, and egg products to the US or Canada. In 2003, the US imported approximately 3,858,822,771 pounds of fresh and processed meat, poultry, and egg products from 23 countries. The import testing program included eight compound classes of veterinary drugs and pesticides, and only two residue violations were found out of the 2,212 samples; both were avermectin residues in fresh beef from Costa Rica. In the imported meat program in Canada in 2003, only a single sample of pork from the US was detected containing sulfamethazine. Honey imported into Canada in 2003 had a low level of antimicrobials detected, but included 27 detections of chloramphenicol-contaminated honey imported from India and six from the US. Nitrofurantoin-containing honey was detected in imports from Australia and Turkey.

Residues in Milk and Dairy Products

Milk that is contaminated with antimicrobials is considered a public health hazard because of adverse reactions and antimicrobial resistance. Antimicrobials are known to interfere with the manufacture of dairy products; concentrations of 1 ppb delay starter activity for cheese, butter, and yogurt. Antimicrobials decrease the acid and flavor production associated with butter manufacture, and they reduce the curdling of milk and cause improper ripening of cheeses.

The odds that a violative antimicrobial residue will be found in bulk tank milk increases with increasing milk production and an increase in the somatic cell count (SCC) status of the herd. Larger herds may have more problems with management as there are typically more employees responsible for treatments and more cow records to maintain. The SCC is an indicator of the prevalence of mastitis within a herd and such infections are routinely treated with antimicrobials in order to lower the SCC to acceptable levels (Ruegg and Tabone, 2000; Saville, et al., 2000). In the US, the National Milk Drug Residue Data Base is a voluntary industry reporting program. Mandatory reporting is required by state regulatory agencies under the National Conference on Interstate Milk Shipments. The Pasteurized Milk Ordinance requires all bulk milk tankers to be sampled and analyzed for animal drug residues before the milk is processed. In ad-

dition, a minimum of four samples from pasteurized fluid milk and milk products must be tested from each plant every six months and each producer must be tested at least four times every six months.

In the US in 2003, 4,456,141 tests were conducted on 4,382,974 samples and 3,246 were positive (NCIMS, 2003). Of the positive samples, 1,899 of 3,571,834 were from milk tankers, eight of 54,932 were from pasteurized fluid milk or milk products, and 1,009 of 665,627 were from producer samples. The violations resulted in the discard of 76,370,000 pounds of milk. The majority of residue violations were due to beta-lactam antibiotics and sulfonamides (Table 25.8). The most frequently used residue test was the Charm SL-Beta-lactam (2,300,000), followed by the Delvotest P5 Pack Beta-lactam (624,000), IDEXX SNAP Beta-lactam (451,000), and Charm II Tablet Competitive Beta-lactam (334,000) tests.

In Canada, regulation of milk and dairy products is done on a provincial basis. Drug residue statistics from individual provinces are not available. In the 2002/2003 federal program, the CFIA tested 3,577 milk and cheese products with no antimicrobial or sulfonamide violations detected.

Drug residues in milk are tested for by several methods, including microbial growth inhibition assays, microbial receptor assays, receptor binding assays, immunologic assays, enzymatic assays, and chromatographic analysis (Mitchell et al., 1998). Because of the problems and penalties associated with antimicrobial residues in milk and dairy products, a number of rapid antimicrobial screening tests have been developed for testing bulk tank or tanker milk. Despite brand names that include the term "cowside", none of the tests are currently validated for testing individual cows. In determining label milk withdrawal times for dairy drugs, the regulatory agencies assume that milk from a treated cow will be commingled with milk from untreated cows, so the MRLs are based on bulk tank concentrations. So if a label withdrawal time is followed, it would not be unusual for a milk sample from a treated cow to test positive while a sample from the commingled milk in the bulk tank would test negative (often humorously referred to as "the solution to pollution is dilution").

The commercially available screening tests have excellent sensitivities for the antimicrobials they are designed to detect and excellent negative predictive values, but they have poor positive predictive values. So a

Table 25.8. Antimicrobial residues in milk and dairy products in the United States in 2003.*

Drug	Total Tests	Positive Tests
Aminoglycosides	1,290	1
Phenolics	201	0
Beta-lactams	4,354,087	3,207
Cloxacillin	317	3
Macrolides	64	0
Neomycin	1,858	2
Sulfadimethoxine	4,478	3
Sulfamethazine	17,466	3
Sulfonamides	66,124	23
Tetracyclines	10,256	4
Total	4,456,141	3,246

*National Milk Drug Residue Data Base

negative test on an individual cow is excellent insurance that a violation on the bulk tank milk will not be detected, but a positive test on an individual cow will not necessarily result in bulk tank drug concentrations above the legal MRL (Gibbons-Burgener et al., 2001). In such cases, discarding a treated cow's milk past the label withdrawal time is unnecessary and wasteful.

However, Sischo et al. (1997) reported that the use of antimicrobial residue screening tests for evaluating an individual cow's milk was associated with a reduction in the risk of residue violations. In addition, the Milk and Dairy Beef Residue Prevention Protocol of the Dairy Quality Assurance Program recommends that milk from individual cows be tested for antimicrobial residues following extra-label use of antimicrobials. Testing milk from treated cows following an appropriate milk-withholding period allows the dairy producer to make informed decisions about milk withholding and reduces the risk of contamination of commingled milk (Andrew, 2000).

In Table 25.9, three commonly used "cowside" screening tests are compared according to their sensitivities for specific antimicrobials against the MRL values in the US, Canada and the European Union. For some tests, the sensitivity is far below the legal MRL (e.g., ceftiofur, cephalixin) and can result in "sub-violative positives" with unnecessary milk discard. For others, the sensitivity is above the legal MRLs, resulting in false negatives (e.g., cloxacillin, erythromycin). In Canada, legal MRLs have not been established for some drugs that are approved for use in lactating dairy

Table 25.9. Sensitivities of milk residue screening tests compared to United States, Canada, and European Union MRLs.

Drug	Test	Sensitivity (ppb)	US MRL (ppb)	CDN MRL (ppb)	EU MRL (ppb)
Amoxicillin	Charm Cowside	6	10	NE	4
	Delvotest SP	2			
	IDEXX SNAP B-lactam	7.3			
Ampicillin	Charm Cowside	5	10	10	4
	Delvotest SP	2-3			
	IDEXX SNAP B-lactam	5.8			
Ceftiofur	Charm Cowside	50-100	50	100	100
	Delvotest SP	<50			
	IDEXX SNAP B-lactam	5.4			
Cephapirin	Charm Cowside	10	20	20	10
	Delvotest SP	5			
	IDEXX SNAP B-lactam	11.7			
Chlortetracycline	Charm Cowside	300	300	NE	100
	Delvotest SP	100-150			
	IDEXX SNAP Tetracycline	30			
Cloxacillin	Charm Cowside	30-50	10	NE	30
	Delvotest SP	15			
	IDEXX SNAP B-lactam	50			
Dicloxacillin	Charm Cowside	30-50	NE	NE	30
	Delvotest SP	10			
	IDEXX SNAP B-lactam	50			
Erythromycin	Charm Cowside	150	50	50	40
	Delvotest SP	50			
Gentamicin	Charm Cowside	300-400	30	NE	100
	IDEXX SNAP Gentamicin	30			
	Delvotest SP	100-300			
Lincomycin	Charm Cowside	200	150	NE	150
	Delvotest SP	100			
Neomycin	Delvotest SP	100-200	150	NE	500
Novobiocin	Delvotest SP	600	100	NE	100
Oxytetracycline	Charm Cowside	200-300	300	NE	100
	Delvotest SP	100			
	IDEXX SNAP Tetracycline	30			
Penicillin G	Charm Cowside	3-4	5	10	4
	Delvotest SP	2			
	IDEXX SNAP B-lactam	3.0			
Pirlimycin	Charm Cowside	100-200	400	400	100
	Delvotest SP	50			
Polymyxin B	Delvotest P	30	0	4	NE
Sulfonamides	Charm Cowside	25-200	10	10	100
	Delvotest SP	25-50			
Tetracycline	Charm Cowside	100	300	NE	100
	Delvotest SP	100			
	IDEXX SNAP Tetracycline	20			
Tilmicosin	Charm Cowside	80	0	NE	40
Tylosin	Charm Cowside	40-50	50	NE	50
	Delvotest SP	10-20			

NE, no legal maximum residue limit established.

cattle (e.g., oxytetracycline). High levels of natural inhibitors are present in mastitic milk and in colostrum and they can cause false positive results in the microbial growth inhibition assays. Heating milk to 82°C for five minutes inactivates natural inhibitors and can be used to prove false-positive results in the microbial growth inhibition assays. (Kang et al., 2005). High concentrations of milk protein and milk fat can adversely affect antimicrobial residue test performance, but the degree of the effect depends upon the analytical method of the screening test (Andrew, 2000.) Higher concentrations of immunoglobulins and milk protein can also cause false positives with screening tests used on samples from recently freshened heifers or cows (Andrew, 2001).

Effect of Drug Residues in Food on Human Health

Residues of antimicrobial drugs in animals raise special human safety concerns with regard to allergenicity and effects on human intestinal microflora. Ordinary cooking procedures for meat, even to “well-done”, cannot be relied on to inactivate even the more heat-sensitive compounds such as penicillins and tetracyclines. More severe heating, as for canning, or prolonged cooking with moist heat can inactivate the more heat-sensitive compounds, but the nature of the degradation products is unknown in most cases (Moats, 1999). Allergic reactions are manifested in many ways from life-threatening anaphylactic reactions to lesser reactions such as rashes. Although animal drug residues do not cause primary sensitization of individuals because exposures are too low and for short duration, violative residues of animal drugs in food have caused allergic reactions in sensitive individuals. Violative residues of penicillin are the most frequently cited cause of allergic reactions in persons consuming residues. Many other animal drugs, including tetracyclines, sulfonamides and aminoglycosides, can cause allergic reactions in sensitive individuals.

Reports of acute adverse reactions in humans from ingestion of drug residues are rare. Of the few reports that document adverse reactions in people consuming residue-contaminated foods, the overwhelming majority are allergic reactions to penicillin. In reference to these allergic reactions, Burgat-Sacaze et al. (1986) stress the following: (1) Involvement of residues constitutes a very low number of cases (a

small percentage) of food allergies. The major allergens involved are natural food constituents or human food additives. (2) Rashes are the most frequently observed clinical finding; anaphylactic shock has not been reported. (3) In most cases, residues are implicated without sufficient diagnostic evidence. Most suspicions are established as follows: Reaction of hypersensitivity observed following food intake; tests demonstrate that the individual is not allergic to the food eaten but is to some drugs; and hence the possibility of the presence of residues of these drugs in the food is raised without checking the hypothesis. Thus “circumstantial evidence” is often the only criterion and residue involvement is anecdotal in several reports. Nearly all reports of acute adverse reactions to foodborne residues implicate penicillin as the offending agent, and the source of penicillin residues is most often milk or dairy products. These milk residues likely originated from intramammary infusion of penicillin used for the treatment of mastitis (Siegel, 1959). Although a substantial number of farm milk samples have been found to contain small amounts of penicillin, there have been relatively few published reports of adverse reactions from milk residues (Erskine, 1958; Vickers et al., 1958; Zimmerman, 1959; Borrie and Barrett, 1961; Wicher et al., 1969). In all instances, the victims reported a history of penicillin allergy or skin disease unrelated to penicillin allergy. Symptoms varied in intensity from mild skin rashes to exfoliative dermatitis. In an investigation of 252 patients with chronic recurrent urticaria, 70 (27.8%) were determined to be allergic to penicillin by dermal testing. When 52 of these penicillin-allergic patients were restricted to a diet containing no milk or dairy products, 30 experienced remission of symptoms, whereas only two out of a group of 40 patients with chronic urticaria and negative skin tests responded favorably to the milk-free diet (Boonk and Van Ketel, 1982). Many drugs other than penicillin—including other beta-lactams, streptomycin (and other aminoglycosides), sulfonamides, and to a lesser extent, novobiocin and the tetracyclines—are known to cause allergic reactions in sensitive persons; however, with the exception of a single report of a reaction to meat suspected of containing streptomycin residues (Tinkleman and Bock, 1984), there are no reports of foodborne allergic reactions resulting from residues of any drug other than penicillin. Drug allergies are generally considered to be type 1 immune re-

sponses (Coombs and Gell, 1975). These reactions are mediated through IgE and symptoms include anaphylaxis, urticaria, and angioedema.

The commensal bacteria that populate the human gastrointestinal tract provide a barrier to infectious agents, metabolize toxins and carcinogens, produce vitamins, and aid in food digestion. Therapeutic concentrations of some antimicrobials perturb the gastrointestinal ecosystem, causing adverse health effects. Abnormal or soft stools or diarrhea are considered adverse biological effects in the human food safety evaluation. Although soft stools and diarrhea following administration of antimicrobials are generally considered to be indirect biological effects rather than a true toxic effect of the drug, these effects are the result of perturbations of the intestinal microflora and therefore are considered adverse effects of the drug. Further, there exists the potential that overgrowth of pathogenic microorganisms could cause colitis or septic conditions, especially in immunocompromised individuals. Colonization resistance sustained by indigenous anaerobes within the intestine could be compromised, resulting in increased susceptibility to invasion by pathogenic organisms. Metabolic activity of the flora could be altered, resulting in altered metabolism of different compounds. Lastly, populations of antimicrobial-resistant strains of enteric pathogens may emerge as a result of selective pressure by antimicrobials (Boisseau, 1993).

The drug approval agencies are now considering the microbiological effects of antimicrobial residues in new drug submissions. In 2003 the FDA released Guidance Document #152: Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to their Microbiological Effects on Bacteria of Human Health Concern. Similarly, in 2004 the VDD adopted the guidelines of the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (aka VICH): Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: General Approach to Establish a Microbiological ADI (Canadian VDD, 2004). These documents are not regulations but science-based processes drug sponsors may use when they seek approval of an antimicrobial for use in food-producing animals. These guidance documents recommend studies to assess microbiological endpoints, including changes in colonization resistance properties, metabolic activity, antimicrobial resistance patterns, and

number and composition of the microorganisms that constitute the intestinal microflora.

Preventing Residues: The Role of FARAD

The Food Animal Residue Avoidance Databank (FARAD) was established in 1982 as a cooperative project of North Carolina State University, the University of California, the University of Florida, and the US Department of Agriculture's Food Safety and Inspection Service (FSIS) as a way to reduce the rate of residue violations in animal products through education and information. The founding philosophy of FARAD was that information about residue avoidance from all sources should be immediately available from a scientific source. The FARAD was developed as a clearinghouse for information on approved animal drugs, extra-label drug use, and environmental toxins. For this "one-stop shopping" information service to work, the FARAD information was collated into a searchable computer database, with residue and pharmacokinetic data analyzed and interpreted by veterinary pharmacologists and toxicologists. Currently, the FARAD database includes over 1200 drugs and chemicals and over 20,000 pharmacokinetic records extracted from over 11,000 citations. The FARAD system focuses on published pharmacokinetic information such as the biological half-lives, clearance rates, and volumes of distribution for those drugs, pesticides, and environmental contaminants that have the greatest potential for persisting in tissues of livestock. From these pharmacokinetic values, mathematical models are developed from which residue-depletion times can be estimated regardless of the drug dose. For over 20 years the US FARAD centers have been providing accurate and timely information to veterinarians to protect the US food supply. In 1998, 11 countries, including Canada, embraced the concept of "global" FARAD centers with multinational cooperative sharing of data on food animal drugs and residue avoidance. Each member country is responsible for establishing its own permanent national access centers. This international cooperation augments efforts to ensure that withdrawal recommendations and interspecies extrapolations are based on the best scientific information available. In 2002, the Canadian gFARAD began operations at the Western College of Veterinary Medicine at the University of Saskatchewan and the Faculté de

Médecine Vétérinaire at the University of Montreal with support from Agriculture and Agri-Food Canada and Canadian veterinary and producer groups.

When using drugs in an extra-label manner in food animals in the US and Canada, or in the event of animal exposure to pesticides, herbicides, and other toxic chemicals, veterinarians should contact their global Food Animal Residue Avoidance Databank (gFARAD) for withdrawal recommendations. In the United States, veterinarians may call 1-888-USFARAD (1-888-873-2723), send email to FARAD@ncsu.edu or go to www.farad.org. In Canada, veterinarians may call 1-866-CGFARAD (1-866-243-2723), send email to [cgfarad@umontreal.ca](mailto:cgf-arad@umontreal.ca) or go to www.cgf-arad.usask.ca. When contacting a gFARAD center, the veterinarian should be prepared to provide information regarding the brand name and generic name of the drug, the dose, the type and number of animals treated, and the disease condition prompting treatment.

Conclusions

Food safety is one of the most significant issues facing animal agriculture. Consumer concerns about drug and chemical residues continue to erode the demand for animal-derived foods. Globally, concerns over food safety have resulted in disruption of international trade. Formal training in the area of residue prevention has been limited at a time when advances are rapidly reshaping the way that food safety programs operate. The development of rapid immunodiagnostic tests for drug residues has allowed the monitoring of much greater numbers of animal products prior to reaching the food supply. HACCP-like quality assurance programs are in use that require the livestock producer and packer to verify that their animals and animal products are wholesome and free of drug residues. HACCP programs are in place at federally inspected abattoirs. Failure of the veterinary profession and the livestock industry to embrace "farm-to-plate" programs will ultimately undermine the public's confidence in the safety of the food supply. Clearly, at a time when consumer demand for a safe and wholesome food supply has never been greater, the need for national regulatory authorities, the veterinary profession and the livestock industry to assert strong leadership and cooperation for food safety has never been more critical.

Bibliography

- Andrew SM. 2000. Effect of fat and protein content of milk from individual cows on the specificity rates of antibiotic residue screening tests. *J Dairy Sci* 83:2992.
- Andrew SM. 2001. Effect of composition of colostrum and transition milk from Holstein heifers on specificity rates of antibiotic residue tests. *J Dairy Sci* 84:100.
- Boisseau J. 1993. Basis for the evaluation of the microbiological risks due to veterinary drug residues in food. *Vet Microbiol* 35:187.
- Boonk WJ, Van Ketel WG. 1982. The role of penicillin in the pathogenesis of chronic urticaria. *Brit J Dermatol* 106:183.
- Borrie P, Barrett J. 1961. Dermatitis caused by penicillin in bulked milk supplies. *Brit Med J* 2:1267.
- Burgat-Sacaze V, et al. 1986. Toxicological significance of bound residues. In: Rico A (ed). *Drug Residues in Animals*. Orlando, FL: Academic Press.
- Canadian Food Inspection Agency (CFIA). 2003. National Chemical Residue Monitoring Program annual report. <http://www.inspection.gc.ca/english/fssa/microchem/resid/reside.shtml#resid>.
- Canadian Veterinary Drugs Directorate (VDD). 2004. Studies to evaluate the safety of residues of veterinary drugs in human food: general approach to establish a microbiological ADI. VICH GL 36. http://www.hc-sc.gc.ca/dhp-mps/legislation/vet/guide-ld/vich/ich-cih_gl_36_e.html.
- Coombs RRA, Gell PGM. 1975. Classification of allergic reactions responsible for clinical hypersensitivity and disease. In: Gell PGM (ed). *Clinical Aspects of Immunology*. Oxford: Blackwell Scientific.
- Erskine D. 1958. Dermatitis caused by penicillin in milk. *Lancet* 1:431.
- Fitzpatrick SC, et al. 1995. Dietary intake estimates as a means to the harmonization of maximum residue levels for veterinary drugs. I. Concept. *J Vet Pharmacol Ther* 18:325.
- Fitzpatrick SC, et al. 1996. Dietary intake estimates as a means to the harmonization of maximum residue levels for veterinary drugs. II. Proposed application to the free trade agreement between the United States and Canada. *Reg Tox Pharm* 24:177.
- Huber WG. 1971. The impact of veterinary drugs and their residues. *Adv Vet Sci Comp Med* 15:101.
- Gibbons-Burgener SN, et al. 2001. Reliability of three bulk-tank antimicrobial residue detection assays used to test individual milk samples from cows with mild clinical mastitis. *Am J Vet Res* 62:1716.
- Kang JH, et al. 2005. False-positive outcome and drug residue in milk samples over withdrawal times. *J Dairy Sci* 88:908.
- Mitchell JM, et al. 1998. Antimicrobial drug residues in milk and meat: causes, concerns, prevalence, regulations, tests, and test performance. *J Food Prot* 61:742.
- Moats WA. 1999. The effect of processing on veterinary residues in foods. *Adv Exp Med Biol* 459:233-41.
- NCIMS. 2003. National Milk Drug Residue Data Base Fiscal Year 2003 Annual Report. <http://vm.cfsan.fda.gov/~ear/milkrp03.html>.

- Paige JC, et al. 1999. Federal surveillance of veterinary drugs and chemical residues (with recent data 1992-1996). *Vet Clin North Am* 15(1).
- Ruegg PL, Tabone TJ. 2000. The relationship between antibiotic residue violations and somatic cell counts in Wisconsin dairy herds. *J Dairy Sci* 83:2805.
- Saville WJ, et al. 2000. Association between measures of milk quality and risk of violative antimicrobial residues in grade-A raw milk. *J Am Vet Med Assoc* 217:541.
- Siegel B. 1959. Hidden contacts with penicillin. *Bull WHO* 21:703.
- Sischo WM, et al. 1997. Implementing a quality assurance program using a risk assessment tool on dairy operations. *J Dairy Sci* 80:777.
- Tinkleman DG, Bock SA. 1984. Anaphylaxis presumed to be caused by beef containing streptomycin. *Ann Allergy* 53:243.
- US FDA Center for Veterinary Medicine. 2003. Evaluating the safety of antimicrobial new animal drugs with regard to their microbiological effects on bacteria of human health concern. Guidance Document 152. <http://www.fda.gov/cvm/guidance/fguide152.doc>.
- USDA Food Safety and Inspection Service (FSIS). 2003. National residue program data. http://www.fsis.usda.gov/Science/2003_Red_Book/index.asp.
- Vickers HR, et al. 1958. Dermatitis caused by penicillin in milk. *Lancet* 1:351.
- Wicher K, et al. 1969. Allergic reaction to penicillin. *J Am Med Assoc* 208:143.
- Zimmerman MC. 1959. Chronic penicillin urticaria from dairy products proved by penicillinase cures. *Arch Dermatol* 79:1.

Regulation of Antibiotic Use in Animals

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Approval and licensing of antimicrobials for use in animals, particularly food-producing animals, is a complex process that strives to ensure that products are effective and safe. It also involves management of the risks of adverse effects from antimicrobial use. The traditional risk areas that are considered in the approval of animal antimicrobial products include the following:

- Harm to organisms inadvertently exposed (environmental safety)
- Harm to people directly exposed (human occupational safety)
- Harm to people indirectly exposed through consumption or exposure to food products of animal origin (human food safety)
- Harm in treated animals (target animal safety)
- Failure to achieve claims (efficacy)

Within these risk areas, some of the hazards and exposure pathways are well known and the methodology for collecting and analyzing these data is well characterized (Miller and Flynn, 2000). Other hazards, such as the transfer of antimicrobial resistance to human bacterial pathogens, are of more recent concern and the focus of new policies.

The drug sponsor or party that is requesting approval to market a veterinary drug applies for approval to the relevant regulatory authority. It is the responsibility of the sponsor to provide data demonstrating that the product is safe and effective and is manufactured to appropriate standards of quality and purity. It is the responsibility of regulators to evaluate the information and data provided, consider management options, and impose conditions on marketing approval to reduce risks.

Antimicrobials undergo comprehensive, in-depth testing prior to receiving marketing approval. Many antimicrobials produced for animals are marketed globally and many countries have similar data requirements. In the past ten years, concerted effort has been expended to promote international harmonization of animal drug regulatory requirements. The International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) was established in 1996 under the auspices of the Organization International Epizooties (OIE) (<http://vich.eudra.org>). VICH has developed and harmonized study protocols, criteria, and standards for the registration of new pharmaceutical and immunological veterinary products and standards for post-marketing surveillance activities and reporting. Original government and industry participants from the European Union, Japan and the United States have been joined, over the years, by observers and interested parties from Canada, Australia, New Zealand, CAMEVET (a Latin American government-industry association) and the Association of Veterinary Biologics Companies. The International Federation of Animal Health serves as the VICH Secretariat. By spring 2005, the VICH finalized 30 guidance documents. (<http://vich.eudra.org/html/guidelines.htm>)

This chapter will describe the documentation requirements for approval of veterinary antimicrobial agents and identify variations in the United States, New Zealand/Australia, and the European Union.

In the United States, the regulatory authority for approval of veterinary drugs is the Food and Drug Administration (FDA) Center for Veterinary Medicine. The legal basis is set in the Federal Food, Drug and Cosmetic Act of 1996 as amended and associated regulations. In order for a veterinary drug to be legally

marketed in the US, it must have an approved new animal drug application.

In the European Union (EU), the legal basis for approval of veterinary medicinal products is set in the European Community legislation, and dossier requirements are detailed in Directive 2001/82/EC of the European Parliament and of the Council as amended by Directive 2004/28/EC (EUDRALEX Volume 5). For setting maximum residue limits, the legal basis is set in European Council Regulation 2377/90, and detailed guidance of this process is provided in the EUDRALEX Volume 8. EUDRALEX is a series publication governing medicinal products in the European Union.

Application for marketing authorization can be processed by different procedures in the European Union. In the centralized procedure, which is intended for new chemical entities and innovative products, the Committee for Medicinal Products for Veterinary Use (CVMP) of the European Medicines Agency (EMA) carries out the scientific assessment. If a positive opinion is reached by the CVMP and the EU Commission, a decision is given to authorize the product. This decision is binding for all 25 member states. Other procedures to obtain marketing authorization include the mutual recognition procedure and the decentralized procedure, which involve several or all member states. If no agreement is reached on the authorization, or a member state or the EU Commission considers that there is a potentially serious safety issue, applications and marketing authorizations can be referred to the CVMP.

In New Zealand, the statutory basis for registration of veterinary medicines is provided in the Agricultural Compounds and Veterinary Medicines (ACVM) Act of 1997. The ACVM Group of the New Zealand Food Safety Authority (NZFSA) is the registration authority in New Zealand. Veterinary medicines containing antibacterial ingredients commonly referred to as antibiotics must be registered under the ACVM Act and conditions are imposed to regulate their importation, manufacture, sale and use.

Demonstration of Quality

Sound quality is essential for the safety and efficacy of a veterinary medicinal product. Adequate quality of the veterinary medicinal product ensures batch-to-

batch consistency and that the product fulfills the established specifications to the end of the authorized shelf life. For these reasons, all veterinary medicinal products should be manufactured to the appropriate quality and purity and produced in compliance with the provisions of Good Manufacturing Practices (GMPs). Conditions on market approvals are set by regulators to ensure safety and efficacy, given the product specifications and approved uses. The company's ongoing ability to produce the product to approved specifications becomes a cornerstone in monitoring and compliance.

Quality was the first discipline on which international harmonization was achieved via the VICH process, although many regional quality guidelines continue to exist. For reference, the reader should also see VICH guidance documents GL1-GL5, GL8, GL10-11, and GL17-18.

Demonstration of Safety

Before an animal drug can be marketed, the sponsor must demonstrate, using appropriate testing methods, that the drug is safe for use under the conditions prescribed, recommended, or suggested in the proposed labeling. The requirements for demonstration of safety can be separated into environmental safety, user safety, human food safety (consumer safety) and target animal safety. A package of pharmacological and toxicological data, based on studies in laboratory animals, is required for all pharmacologically active substances in antimicrobial veterinary products. These data form the basis for user safety, food safety, and target animal safety. The requirements are more extensive if the substance is intended for use in food-producing animals, as described in the section on Human Food Safety.

Environmental Safety

Environmental protection goals includes minimum risk to water (ground and drinking), air, soil, plant, animal, and nonpathogenic microbial species that may be exposed. Standards are set by either legislation or policy by the pertinent regulatory body in each country. An exposure threshold approach is generally used to determine when environmental fate and effect studies are needed. Environmental studies are not necessary for compounds that have limited environmental introductions, for example, antimicrobial products

that are used to treat individual dogs. When an environmental assessment is required, the drug sponsor conducts laboratory-based toxicity studies with invertebrates, plants and microorganisms representative of the environmental compartment of concern. The no-observed-effect level, or the MIC in the case of microbes, is divided by a safety factor to arrive at a Predicted Environmental No-Effect Concentration (PNEC). The predicted environmental concentration (PEC) is calculated for the drug and compared to the PNEC. When the PEC/PNEC ratio is less than one, significant environmental effects are not predicted to occur due to the use of the animal drug product (Miller and Eirkson, 1997).

VICH has developed extensive guidance to assess the potential for veterinary medical products to affect non-target species in the environment, including both aquatic and terrestrial species. Evaluation of environmental effects is carried out in two phases. Phase I guidance describes criteria for determining whether an environmental impact assessment should be undertaken (VICH GL6, 2001). If the exposure limits set are exceeded in Phase I, the Phase II assessment is needed to obtain data on environmental fate, metabolism, and toxicity of the active substance, using the test methods described in the Phase II guideline (VICH GL38, 2003). The VICH Phase II guidance contains sections for aquaculture, intensively reared terrestrial animals, and pasture animals. Intensive animal operations are those in which animals are kept and raised in confined situations, in a relatively small land area. Beef cattle, dairy cattle, pigs, chickens, and turkeys are examples of species that may be reared in an intensive terrestrial system. The use of antimicrobial agents in animal production has facilitated confinement housing and allowed large numbers of animals to be produced on small land areas. On the basis of the environmental studies, the applicant proposes appropriate risk management measures to mitigate potential adverse effects to the environment.

User Safety

A veterinary medicinal product must not enter food-stuffs in a way that endangers consumers, and must also be safe for persons who administer the product to animals. For this reason, user safety is evaluated during the authorization process. The most common concern regarding antimicrobial products is the risk of hypersensitivity reactions. Since some injectable prod-

ucts cause severe tissue irritation, accidental self-injection is also of concern.

Safety of the veterinary drug product to the user is considered prior to marketing approval by evaluating the toxicity profile of the drug, the route of administration, the packaging, and instructions to the veterinarian or animal caregiver. Acceptable risk management procedures include warning and contraindication statements on the label, including black-box labeling if the toxic effect to humans is potentially severe. Also, the product information should contain all necessary instructions and warnings for safe use. In some situations, use of the product may be limited to veterinarians as a safety precaution. The recently developed EU guidelines for risk assessment of user safety (EMA 2005) helps applicants and risk managers make valid operator safety assessments.

Human Food Safety

Assessment of Hazards from Veterinary Drug Residues

The hazards associated with the consumption of food containing residues of veterinary drugs used in food-producing animals are generally assessed in laboratory animals treated with the drugs (VICH GL33, 2004). To determine the food safety of residues of an antimicrobial substance, the drug sponsor conducts a standard battery of animal-based toxicology tests. The battery of animal studies for antimicrobial agents to be used in food-producing animals includes tests for repeat-dose toxicity (VICH GL31, 2002 and GL37, 2004), reproduction toxicity (VICH GL22, 2001), developmental toxicity (VICH GL32, 2002), genotoxicity (VICH GL23, 2001), and effects on human intestinal flora (VICH GL36, 2004).

These tests must provide adequate data to ensure human food safety. The toxicology studies are designed to determine the minimum dose that causes a toxic effect and the maximum dose that causes no observed adverse effect (NOEL). These endpoints are then used to calculate an acceptable daily intake (ADI).

Repeat-dose toxicity testing is used to define toxic effects based on repeated and/or cumulative exposures to the compound and its metabolites, the incidence and severity of the effect in relation to dose and duration of exposure, doses associated with toxic and biological responses, and a NOEL. These studies are performed in a sensitive animal species that is appropriate for the studies, with the rat and the dog being the primary default species. Ninety-day toxicity testing is first

performed in order to identify target organs and toxicological endpoints, and to provide data to help determine appropriate doses and species to be used in repeat-dose chronic toxicity testing (VICH GL31, 2002). Next, chronic toxicity testing is performed to define toxic effects based on long-term exposure to the compound or its metabolites, identify target organs and toxicological endpoints in relation to dose or duration of exposure, determine dosages associated with these responses, and establish a NOEL (VICH GL37, 2004).

Multi-generation reproduction studies are designed to detect any effect on mammalian reproduction (VICH GL22, 2001). These include effects on male and female fertility, mating, conception, implantation, ability to maintain pregnancy to term, parturition, lactation, survival, growth and development of the offspring from birth through weaning, sexual maturation, and the subsequent reproductive function of the offspring as adults. The objective of this testing is to evaluate risks to reproduction from long-term, low-dose exposures such as may be encountered with veterinary drug residues in food. Thus, a multi-generation study, in which drug effects are examined through more than one generation, is considered the most appropriate model. The study of more than one generation allows detection not only of any effects on adult reproduction, but also effects on subsequent generations due to exposure *in utero* and in the early postnatal period. These types of studies are not designed to detect developmental abnormalities (see below). Multi-generation studies determine the dose at which any effects on reproduction occur, and identify the dose or doses clearly showing no adverse effects. The majority of these studies are conducted in the rat for two generations.

Developmental toxicity studies are designed to detect any adverse effects on the pregnant female and development of the embryo and fetus consequent to exposure of the female, from implantation through the entire period of gestation (VICH GL32, 2002). Effects may include enhanced toxicity relative to that observed in non-pregnant females, embryo-fetal death, altered fetal growth, and structural changes to the fetus. The testing begins with rat studies and if teratogenicity is observed, no further testing is required. However, if the ADI will be based on the teratogenic endpoint, then developmental toxicity tests should be performed in a second species. Also, if equivocal results are achieved with the rat studies, testing in a sec-

ond species, preferably the rabbit, should be conducted. Teratogenicity for this purpose is defined as producing a structural change in the fetus that is considered detrimental to the animal, which may or may not be compatible with life (VICH GL32, 2002).

A battery of genotoxicity tests is used to identify substances that have the capacity to damage the genetic information within cells (VICH GL23, 2001). Since many carcinogens have a genotoxic mode of action, substances that are genotoxic are regarded as potential carcinogens. Those that cause genetic damage in germ cells also have the potential to cause reproductive or developmental effects. The VICH guideline GL23 recommends a standard battery of three tests to screen for the genotoxicity of veterinary drugs. These include a test for gene mutation in bacteria, an *in vitro* test for chromosomal effects in mammalian cells, and an *in vivo* test for chromosomal effects in rodent hematopoietic cells (VICH GL23, 2001). However, some antimicrobials are toxic to the strains used to test for gene mutation in bacteria. In that case, it is recommended that the bacterial test be performed using concentrations up to the limit of cytotoxicity, supplemented with an *in vitro* test for gene mutation in mammalian cells (VICH GL23, 2001).

Carcinogenicity testing is performed for those compounds that are suspected to have carcinogenic potential. Carcinogenic potential is assessed by evaluating the structure of the parent compound, the results of genotoxicity testing, data from chronic toxicity studies and any other relevant information. The VICH guideline on carcinogenicity testing recommends that testing be done by bioassays (VICH GL28, 2002). Carcinogenicity bioassays with oral dosing consisting of a two-year rat study and an 18-month mouse study are generally required. Specific details for carcinogenicity studies may be found in the Organization for Economic Cooperation and Development (OECD) Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies at [http://www.oelis.oecd.org/olis/2002doc.nsf/43bb6130e5e86e5fc12569fa005d004c/b4c177bd1b70d6c4c1256c000054ced6/\\$FILE/JT00130828.DOC](http://www.oelis.oecd.org/olis/2002doc.nsf/43bb6130e5e86e5fc12569fa005d004c/b4c177bd1b70d6c4c1256c000054ced6/$FILE/JT00130828.DOC).

The various toxicological tests are designed to determine the dose at which the compound produces an adverse effect and a dose which produces a NOEL. Once the NOEL is established for all the toxicity endpoints, the most sensitive effect in the species most predictive of man is identified. This NOEL is divided

by a safety factor to account for uncertainty in extrapolating from animals to man and for variability, i.e., the difference among individuals, to calculate an ADI for drug residues. The ADI represents the amount of drug residues that can be safely consumed by an adult daily for a lifetime (Tollefson and Miller, 2000; VICH GL36, 2004.) Sometimes it is also possible to set a pharmacological NOEL on the basis of pharmacological effects of the substance. Based on this NOEL, a pharmacological ADI is determined.

Additional Food Safety Considerations for Antimicrobial Drugs

Antimicrobial drug residues may disrupt the human gastrointestinal flora and increase the population of resistant bacteria. Therefore, a microbiological ADI needs to be established for antimicrobial substances. VICH GL36 (2004) outlines how to determine the need for establishing a microbiological ADI, recommends test systems and methods for determining NOELs for the endpoints of health concern, and recommends a procedure to derive a microbiological ADI. For additional information, Cerniglia and Kotarski published a review in 1999 describing a variety of in vitro and in vivo test systems to study the effects of residues from veterinary antimicrobials on the human gastrointestinal tract (Cerniglia and Kotarski, 1999).

The risk of transfer of resistant bacteria or resistance determinants from foodstuffs of animal origin to humans is potentially a major food safety problem. Risk of this transfer may be assessed for all antimicrobial products intended for use in food-producing animals in accordance with VICH GL27 (2003). See the section on "Management of Antibacterial Resistance" for detailed information.

For some classes of antimicrobials, such as beta-lactam antibiotics, immunotoxicity testing is used to assess the potential for the drug to elicit an allergic reaction in sensitive individuals (VICH GL33, 2004).

Setting Maximum Residue Limits (MRLs) and Establishment of Withdrawal Periods

The lowest pharmacological, toxicological and microbiological ADI provides the basis for determining the maximum residue limits (MRLs). In addition to the safety factors used to calculate the ADI, estimates of daily intake of specific food commodities are generally set conservatively high. Data are also needed on

absorption, distribution, metabolism, and excretion in the target animal species. The marker residue is also identified in the residue studies and is later used for setting the withdrawal period and in residue surveillance.

The MRL is set for each food commodity (e.g. muscle, fat, kidney, liver, milk, eggs, and honey) so that the total consumption of residues falls below the ADI, assuming that all food contains the residue of the drug under consideration and the person consumes the entire amount of each of the commodities. If the intended use of the product is the treatment of milk-producing animals, the MRL in milk must be set low enough to guarantee that starter cultures are not disturbed even if the pharmacological and toxicological data together with the microbiological data on gastrointestinal flora could allow a higher MRL for milk.

The MRL values are established for the active substance. In contrast, the withdrawal periods are set for each veterinary medicinal product because the characteristics of the product have an effect on the behavior of the active substance in the body. The withdrawal period is established for each target animal species using the recommended route of administration, maximum dose, and maximum duration of treatment. If a statistical method is used to establish the withdrawal periods, the marker residue concentrations should be analyzed at different time points using adequate numbers of animals per time point. In the European Union, there are published guidelines on the statistical methods for setting withdrawal periods for tissues and milk (EMA 1995, 1998.)

Target Animal Safety

Demonstration of safety to the animal targeted for the drug is achieved by testing cumulative effects of the drug so as to demonstrate that it does not have an adverse effect on the treated animals. The focus for target animal safety is on the effective and toxic doses and the margin of safety which is represented by the difference between the two (US FDA, 1989). The data required to demonstrate the safety of an animal drug in target animals depends upon the amount of historical information that is available on the subject drug, especially the toxicity studies conducted in laboratory animals. Other information that can be used to determine target animal toxicity study requirements includes pharmacokinetic data, pharmacodynamic indices, metabolism studies, treatment regimen, etc.

Animals used for testing in target animal safety studies should represent the species in which the drug will be used. They should be free of disease and not exposed to environmental conditions that could interfere with the purpose or conduct of the study.

In the EU, the approach to target animal safety testing is more flexible than in the United States. According to the existing guideline (EUDRALEX Volume 7AE2a), overdoses used in the studies need to be justified, taking into account toxicological data from laboratory animal studies and intended use of the product (dose, dosing interval, and duration of treatment.) Based on the results of the target animal studies, information on signs of overdosing and adverse reactions should be included in the Summary of Product Characteristics (SPC) and product literature.

Demonstration of Efficacy

The efficacy studies include preclinical and clinical studies. The number and types required to demonstrate that the antimicrobial drug is effective at the proposed dose or dose range is dependent upon several factors. For example, the proposed intended uses or conditions of use for the antimicrobial, the availability of information concerning the active ingredient of the drug, and whether the antimicrobial is conducive to in vitro testing or data extrapolation are all issues that determine the types of studies required. The drug sponsor must provide a sufficient number of studies of adequate quality and persuasiveness to permit qualified experts to determine whether an animal drug is effective. The quality of a study's design and conduct includes factors of rigor, statistical power, and scope.

Preclinical studies, including pharmacokinetic and pharmacodynamic data, are usually generated to establish an appropriate dosage regimen necessary to ensure the efficacy of the antimicrobial product. Important information about the drug includes the mode of action, the spectrum of antimicrobial activity of the substance, and identification of bacterial species that are naturally resistant to the antimicrobial product studied. Important pharmacodynamic information includes antimicrobial minimum inhibitory concentrations for the target pathogen(s), determination of whether the antimicrobial exerts time or concentration-dependent killing activity, and evaluation of activity at the site of infection. Information is

also needed on secondary pharmacological effects that could, for example, provide explanations on mechanisms of adverse drug reactions. Important pharmacokinetic information includes bioavailability applicable to the route of administration; concentration of the active antimicrobial in the serum and at the site of infection; its volume of distribution in the treated animal; metabolism, including active metabolites; and excretion routes. Pharmacokinetic/pharmacodynamic (PK/PD) modeling can be used to identify potential dosing schemes. These can then be confirmed by dose confirmation studies (EMA, 2001a).

Definitive proof of the efficacy of an antimicrobial product is based upon a demonstration of effectiveness in clinical trials. VICH has established Good Clinical Practice guidance (VICH GL9, 2001) that provides information on the design and conduct of clinical studies of veterinary drugs in the target species. The goal is to ensure the accuracy, integrity, and correctness of the data submitted to the regulatory authority for product registration. The guidance sets out detailed requirements for the clinical investigator, study monitor, and drug sponsor, including instructions on study design, animal selection, animal housing and feeding, and study treatments. Emphasis is placed on developing a comprehensive study protocol in order to help ensure that a well-designed study is developed and executed (VICH GL9, 2001). The EU has specific guidelines for efficacy studies for antimicrobial products; these provide further instructions on how to conduct efficacy trials (EMA, 1999a; EMA, 2001a). Once the trial results are assessed, the essential data for proper use are collected in the Summary of Product Characteristics (SPC), which includes information on pharmacodynamics (including resistance), pharmacokinetics, target animal species, indication, posology, contraindications, warnings, and instructions for proper use (EMA, 2001b.)

The regulatory authority responsible for the approval of veterinary medicinal products evaluates all data submitted in the application and applies conditions on the approval in order to manage the risks associated with the use of the products. The risk management strategies generally used by regulators include approving the product for prescription use only, limiting the approved uses to certain species or market classes of animals, specifying withdrawal periods to manage the risks from residues of veterinary drugs in food, and specifying safety labeling requirements.

Post-Market Monitoring and Compliance

After veterinary drugs are licensed and marketed, monitoring is undertaken to ensure the continued safety and efficacy of the products. Most countries have established systems for reporting adverse drug reactions in animals, monitoring the concentration of residues in animal carcasses and foodstuffs, monitoring the prevalence of antimicrobial resistant organisms, and monitoring the usage of antimicrobials. These post-market surveillance systems provide confidence that veterinary drugs are being used appropriately without unexpected impact on animal or public health. The monitoring results also allow refinement of pre-approval assessment procedures for future applications.

In the EU, the holders of marketing authorization for a veterinary medicinal product must nominate a qualified person to be responsible for pharmacovigilance issues. The requirements are compiled in the EUDRALEX Volume 9. All suspected adverse drug reactions are registered, compiled at set intervals, assessed, and submitted as Periodic Safety Update Reports. In addition, all suspected serious adverse reactions must be reported to the authorities within 15 days. Veterinarians are encouraged to report the reactions they note in their practice either to the marketing authorization holders or to the regulatory authority. The EU pharmacovigilance system covers suspected adverse reactions in animals, humans, and the environment; suspected lack of efficacy; and cases where animal products contain residues although the withdrawal period has been enforced. For antimicrobials, the reports on suspected lack of efficacy are important because these cases may provide early signals on resistance. The systems for reporting adverse drug reactions in New Zealand, Australia, and the United States are similar to those in the EU. Pharmacovigilance is currently a topic undergoing harmonization discussions within VICH.

Monitoring drug residues is discussed in detail in Chapter 25.

Extra-Label Drug Use

Extra-label use of drugs is use of the product in any manner not specified on the label. This includes use in a different species, for a different indication, or

at a dosage different from that on the label. The choice of an alternative product or therapeutic regimen should be based, whenever possible, on the results of valid scientific information demonstrating efficacy for the condition and safety for the species concerned.

In the United States, veterinary oversight is required for the use of approved antimicrobials in an extra-label manner. This direction may only take place within the context of a veterinarian-client-patient relationship (VCPR). A VCPR exists when *all* of the following conditions have been met:

1. The veterinarian has assumed the responsibility for making clinical judgments regarding the health of the animal(s) and the need for medical treatment, and the client has agreed to follow the veterinarian's instructions.
2. The veterinarian has sufficient knowledge of the animal(s) to initiate at least a general or preliminary diagnosis of the medical condition of the animal(s). This means that the veterinarian has recently seen and is personally acquainted with the keeping and care of the animal(s) by virtue of an examination of the animal(s) or by medically appropriate and timely visits to the premises where the animal(s) are kept.
3. The veterinarian is readily available for follow-up evaluation, or has arranged for emergency coverage, in the event of adverse reactions or failure of the treatment regimen.

Extra-label drug use has several constraints or conditions in the US, including:

1. For food animals, such use is not permitted if another drug exists that is labeled for the food animal species and contains the needed ingredient, is in the proper dosage form, is labeled for the indication, and is clinically effective.
2. Extra-label drug use is permitted for therapeutic purposes only when an animal's health is threatened. Extra-label drug use is not permitted for production drugs (e.g., growth promotion).
3. Extra-label drug use is not permitted if it results in a violative food residue, or any residue that may present a risk to public health.
4. Extra-label drug use requires scientifically based drug withdrawal times to ensure food safety.

In the EU, poor availability of veterinary medicinal products, including antimicrobials, is a concern. The requirement to have MRLs for all substances used in food-producing animals has caused problems in the treatment of minor food-producing species, such as rabbits, lactating sheep and goats, for which there is insufficient interest to generate data needed for establishment of MRLs. Situations arise in which the veterinarian does not have products authorized for use in the animal species that must be treated. In such cases, the veterinarian may use other medicinal products off-label, under Directive 2001/82/EC as amended by 2004/28/EC. The rules are more restrictive when the product is to be used in food-producing animals. In this case, the minimum withdrawal periods are 28 days for slaughter, 7 days for milk or eggs, and 500 degree days (the number of days treated at a particular temperature, e.g., 3 days at 4°C = 12 degree days) for fish.

In New Zealand, off-label use is identified as "discretionary use." If the product is for over-the-counter sale and there are no uses that are specifically prohibited, then discretionary use is allowed by non-veterinarians after seeking veterinary advice. Discretionary use by veterinarians is subject to rules similar to those in the United States. Regulations limit discretionary use to animals under the direct care of a veterinarian complying with the Agricultural Compounds and Veterinary Medicines Act of 1997. As in the EU, New Zealand has default withholding times for discretionary use of veterinary drugs. Meat from avian species, monogastric animals, fish, crustaceans, mollusks, lagomorpha and camelids must be withheld from slaughter for 63 days post-exposure. Meat from ruminants, including deer, must be withheld for 91 days. The withholding time for milk is 35 days and for eggs, 10 days. These withdrawal times are conservative and enforce the understanding that discretionary dosing is only to be used when necessary.

Management of Antibacterial Resistance

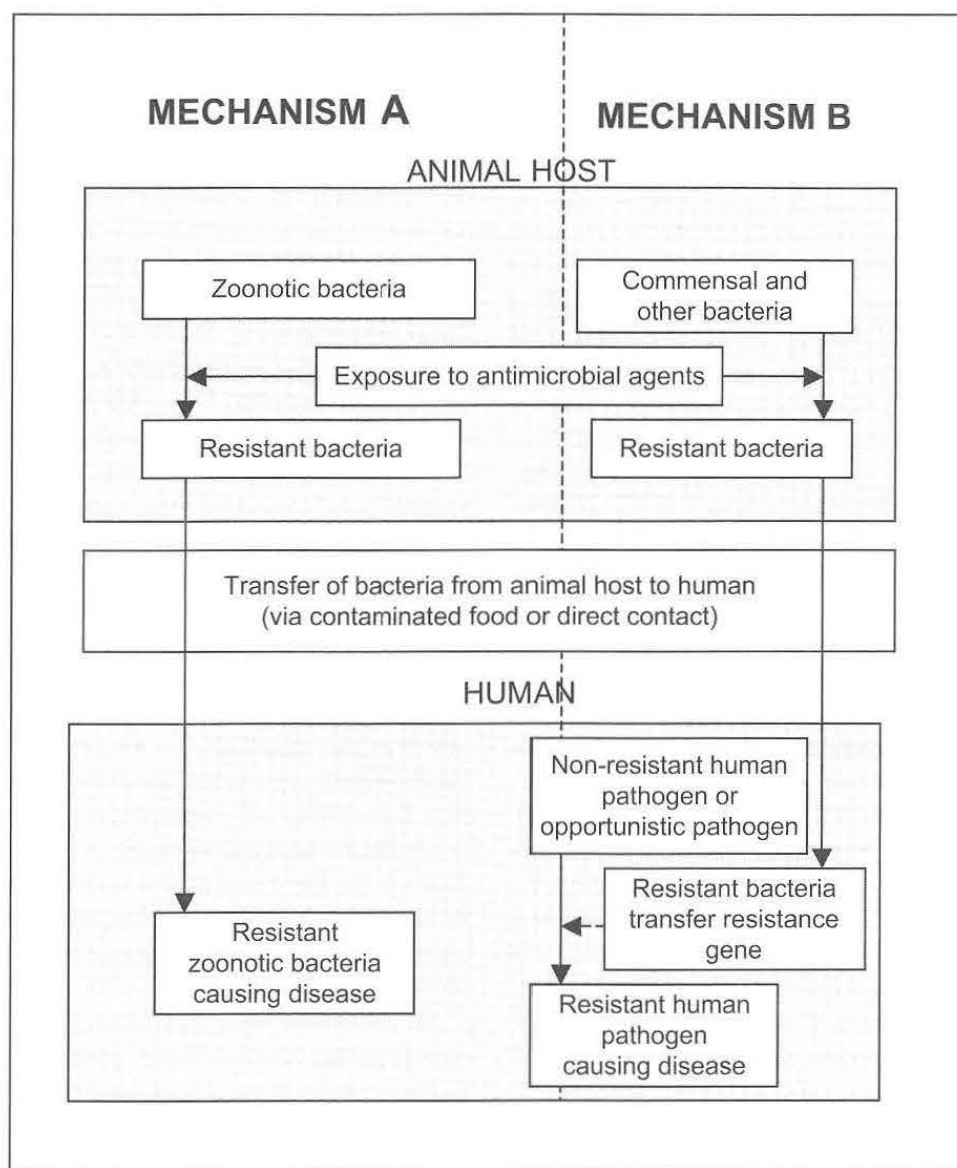
A major concern that should be addressed to ensure the food safety of antimicrobials is the contribution that antimicrobial drug use in food-producing animals plays in the emergence of antimicrobial drug-resistant bacteria. There is general agreement within the scientific community that the development of resistant human pathogenic bacteria results primarily from

the direct use of antimicrobial agents in humans, but also from acquisition of resistant organisms or resistance factors from animal and environmental sources. Antibacterial resistance can develop in both zoonotic bacterial pathogens and commensal bacteria of animal origin that may transfer resistance genes to human pathogenic bacteria or commensals. The two mechanisms are illustrated in Figure 26.1.

Mechanism A is the scenario in which animals are treated with antibacterial products and the zoonotic bacteria present (e.g. *Salmonella* and *Campylobacter* species, *E. coli*, etc.) in the animal, particularly in the gastrointestinal tract, develop resistance. Humans are exposed to the resistant zoonotic bacteria resulting in colonization and possibly disease that is less responsive to antibacterial therapy.

Mechanism B is the more complicated scenario and is thus more hypothetical. In this scenario animals are treated with antibacterial products and commensal bacteria, e.g., *Enterococcus* species, develop resistance. The commensal bacteria referred to in mechanism B are non-pathogenic bacteria in animals that are the same as those in humans or that could, for a short period of time, persist in the gastrointestinal tract of humans. The resistant bacteria either colonize in the human or at least remain long enough to transfer the resistance genetic material to bacteria (possibly pathogenic) in humans.

Global health organizations have been involved with the issue of antimicrobial resistance for several years. In the late 1990s, interest was renewed due to the increasing threat of antimicrobial resistance to human health. In 1997, the World Health Organization (WHO) held a meeting of experts in Berlin, Germany to review the question of whether the use of antimicrobials in animals leads to antimicrobial resistance in humans (WHO, 1997). The experts sought to define potential medical problems that could arise from antimicrobial use in food-producing animals and to make recommendations to address the issue. The group of experts recommended against using antimicrobials for growth promotion, if those antimicrobials are also used in human medicine or can induce cross-resistance to antimicrobials used for human medical therapy. They called for enhanced monitoring of resistance among isolates of enteric bacteria from food animals and food of animal origin. In addition, they recommended managing risk at the producer level through the prudent use of antimicrobials.



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Figure 26.1. Resistance development in zoonotic pathogenic bacteria (Mechanism A) and animal-origin commensal bacteria (Mechanism B).

In June 1998, the WHO held another meeting, in Geneva, Switzerland, to specifically address the use of quinolones in food-producing animals (WHO, 1998). The participants agreed that the use of antimicrobials will select for resistant bacteria and that there is a potential human health hazard from resistant *Salmonella*, *E. coli*, and *Campylobacter* organisms being transferred to humans through the food supply. The experts also concluded that more information was needed to accurately assess the risk associated with the use of these

products in animals, but supported the use of quinolones to treat sick animals when necessary.

The OIE held an international conference on antimicrobial resistance in March 1999 to promote exchange among stakeholders and decision-makers in the human and animal medical fields. The conference served as a forum for countries to report on current regulatory activities and capacities in the detection and quantification of antimicrobial resistance. A second conference took place October 2001 to review

progress achieved in understanding the development of antimicrobial resistance in humans and animals, problems encountered in both human and veterinary medicine, and actions taken by regulatory authorities and others for the containment of antimicrobial resistance (OIE, 2001).

In the EU, antimicrobial substances have been used as feed additives to increase the growth of animals, but this use is being phased out. This action was triggered by the emergence of vancomycin-resistant enterococci (VRE) in chickens and pigs fed avoparcin as a growth promoter (EU, 1997). This was not the first restrictive action in Europe. Sweden, which joined the EU in 1995, prohibited the use of antimicrobial feed additives in 1986. Finland, before joining the EU in 1995, had not allowed the use of spiramycin and tylosin as feed additives because both antimicrobials were used as veterinary drugs. In 1999, the EU prohibited the use of zinc bacitracin, spiramycin, virginiamycin, and tylosin as feed additives (growth promoters). The use of antimicrobials as feed additives will be phased out completely in 2006 except for substances used as coccidiostats or histomonostats (EU, 2003a).

In 1998 the Joint Expert Technical Advisory Committee on Antibiotic Resistance (JETACAR) was appointed and charged by the Australian Minister for Health and Family Services and the Minister for Primary Industries and Energy to examine the issue of antimicrobial resistance from a scientific perspective. The Committee consisted of experts from public health, human and veterinary medicine, molecular biology, and the primary industries. The Committee reviewed the scientific evidence on the link between the use of antibiotics in food-producing animals, the emergence and selection of antibiotic-resistant bacteria, and their spread to humans, and made recommendations for the appropriate future management of antibiotic use in food-producing animals. The final report was issued in September 1999. The JETACAR concluded that there is a hazard to food safety and human health from the use of antibiotics in food-producing animals, which must be weighed against the value of antibiotics to veterinary medicine, food productivity and animal welfare (JETACAR, 1999).

In June 2000 the WHO held an expert consultation with the participation of the Food and Agriculture Organization of the United Nations and the OIE to develop global principles for the containment of antimicrobial resistance in animals intended for food (WHO,

2000). Their purpose was to minimize the negative public health impact of the use of antimicrobial agents in food-producing animals while at the same time providing for their safe and effective use in veterinary medicine. The Principles provide a framework of recommendations to reduce the overuse and misuse of antimicrobials in food animals for the protection of human health. One area stressed in the Principles is the surveillance of antimicrobial resistance and antimicrobial usage.

Two joint FAO/OIE/WHO expert workshops on non-human antimicrobial usage and antimicrobial resistance have been held. The first of these, held in Geneva, Switzerland in December 2003, generated a scientific assessment of antimicrobial resistance. The second was held in Oslo, Norway in March 2004 to discuss risk management strategies. (WHO, 2004a; WHO, 2004b). The workshops recommended that countries expeditiously implement WHO global principles for the containment of antimicrobial resistance in animals intended for foods (WHO, 2000) and follow the OIE guidelines on responsible and prudent antimicrobial use. The Geneva workshop concluded that surveillance of usage of antimicrobials and antimicrobial resistance in foods and animals is necessary for the identification of resistance problems and as a basis for choosing and evaluating interventions to limit the development and spread of resistance (WHO, 2004a).

An additional recommendation arising from both the workshops involved developing either criteria for a list of critical human and veterinary antimicrobial drugs or the actual lists. The OIE held a consultation 26-28 January 2005 in Paris to begin development of the animal criteria. The OIE expert group agreed that the primary criteria should be whether the antimicrobial is used to treat serious disease in animals and whether it is the only available therapy or one of few antimicrobial alternatives. The group also drafted a questionnaire to be sent to member countries for input on the criteria. The WHO held a consultation in February 2005 in Canberra, Australia to develop the criteria for critical antimicrobials for human medical therapy. In order for an antimicrobial to be listed as critical to human medicine, the drug class must be of only one or two alternatives that could be used if resistance developed. In addition, there must be evidence or a reasonable likelihood that resistant bacteria may be transmitted to humans through the food chain

if those critical antimicrobials were used in non-human sources.

Review, Evaluation and Risk Reduction Strategies

The drug sponsor is required to produce documentation in accordance with VICH published guidance on the registration of antimicrobial agents for food-producing animals with respect to antimicrobial resistance (VICH GL27, 2003). The VICH document GL27 provides instructions for gathering basic information on the drug, its mode of action and spectrum of activity, including MICs of target animal pathogens and food-borne and commensal organisms, the mechanism of resistance development, and other related information. The VICH GL27 (2003) does not provide guidance as to how the assessment should be carried out; thus, different approaches have been developed in different regions.

The US FDA published guidance in 2003 that outlines an evidence-based approach to preventing antimicrobial resistance in humans that may result from the use of antimicrobials in animals (US FDA, 2003). The document applies to all antimicrobial drugs intended for use in food-producing animals but only assesses the risk from human exposure through ingestion of antimicrobial-resistant bacteria from animal-derived foods. This is the most significant pathway for human exposure to resistant bacteria that have emerged or been selected for as a consequence of antimicrobial drug use in animals, but it is not the only pathway.

A qualitative approach to risk assessment is recommended in the FDA guidance, although the possibility of quantitative assessments is not excluded. The guidance provides a scientific process for assessing the likelihood that an antimicrobial drug used to treat a food-producing animal may cause an antimicrobial resistance problem in humans consuming milk, eggs, honey, meat, or other edible tissue from an animal. The pathway suggested in the FDA guidance document establishes a three-part system for determining an antimicrobial drug's potential risk to humans if used to treat food-producing animals. The essential components include a release assessment, which determines the probability that resistant bacteria will be present in animals as a result of the use of the drug; an exposure assessment, which gauges the likelihood that humans would ingest the resistant bacteria; and a consequence assessment, which assesses the chances that human exposure to the resistant bacteria would result

Table 26.1. Criteria considered in the US ranking of antimicrobial drugs according to their importance in human medicine.

Critically Important: Antimicrobial drugs which meet both criteria 1 and 2.
Highly Important: Antimicrobial drugs which meet either criteria 1 or 2.
Important: Antimicrobial drugs which meet one or more of criteria 3, 4 or 5.
1. <i>Antimicrobial drugs used to treat enteric pathogens that cause food-borne disease.</i>
2. <i>Sole therapy or one of few alternatives to treat serious human disease, or essential component among many antimicrobials in treatment of human disease.</i> Serious diseases are defined as those with high morbidity or mortality without proper treatment regardless of the relationship of animal transmission to humans.
3. <i>Antimicrobials used to treat enteric pathogens in non-food-borne disease.</i>
4. <i>No cross-resistance within drug class and absence of linked resistance with other drug classes.</i> Absence of resistance linked to other antimicrobials makes antimicrobials more valuable.
5. <i>Difficulty in transmitting resistance elements within or across genera and species of organisms.</i> Antimicrobials to which organisms have chromosomal resistance would be more valuable than those whose resistance mechanisms are present on plasmids and transposons.

in adverse human health consequences. In this context, these are situations in which a physician has difficulty treating a person with an antimicrobial drug because the bacteria infecting the person had acquired resistance to the drug and that resistance came from use of the drug in animals.

For assessment of exposure, the evaluation considers both the frequency of bacterial contamination (e.g., *Salmonella*) of food products and *per capita* consumption of animal-derived food categories from treated animals. The consequence assessment involves placing the drug into "critically important", "highly important" and "important" categories based on the usefulness of the drug in food-borne infections, availability of alternative therapies, the ease with which such resistance develops, and other factors. See Table 26.1 for the US criteria used to categorize the drugs based on importance to human medical therapy and Table 26.2 for examples of drug categorization in New Zealand and the United States.

The risk estimation integrates the three components and classifies the drug as high, medium, or low risk to human health (Figure 26.2).

Table 26.2. Examples of antimicrobial ranking according to importance in human medicine in New Zealand (NZ) and the United States (US).*

Critically Important High Concern	Highly Important Medium Concern	Important Low Concern
Aminoglycosides (NZ)	Aminoglycosides (US)	Amoxicillin, ampicillin (NZ)
3rd-generation cephalosporins (US), 3rd- & 4th-generation cephalosporins (NZ)	Amoxicillin, ampicillin (US)	
Glycopeptides (NZ)	Bacitracin (NZ)	1st- & 2nd-generation cephalosporins (US)
Fluoroquinolones (NZ & US)	4th-generation cephalosporins (US)	Monobactams (US)
Macrolides and lincosamides (NZ & US)	Glycopeptides (US)	Quinolones (US)
Streptogramins (NZ)	Streptogramins (US)	Tetracyclines (NZ)
	Tetracyclines (US)	

*The criteria countries use to categorize veterinary antimicrobials as to their importance in human medicine differ.

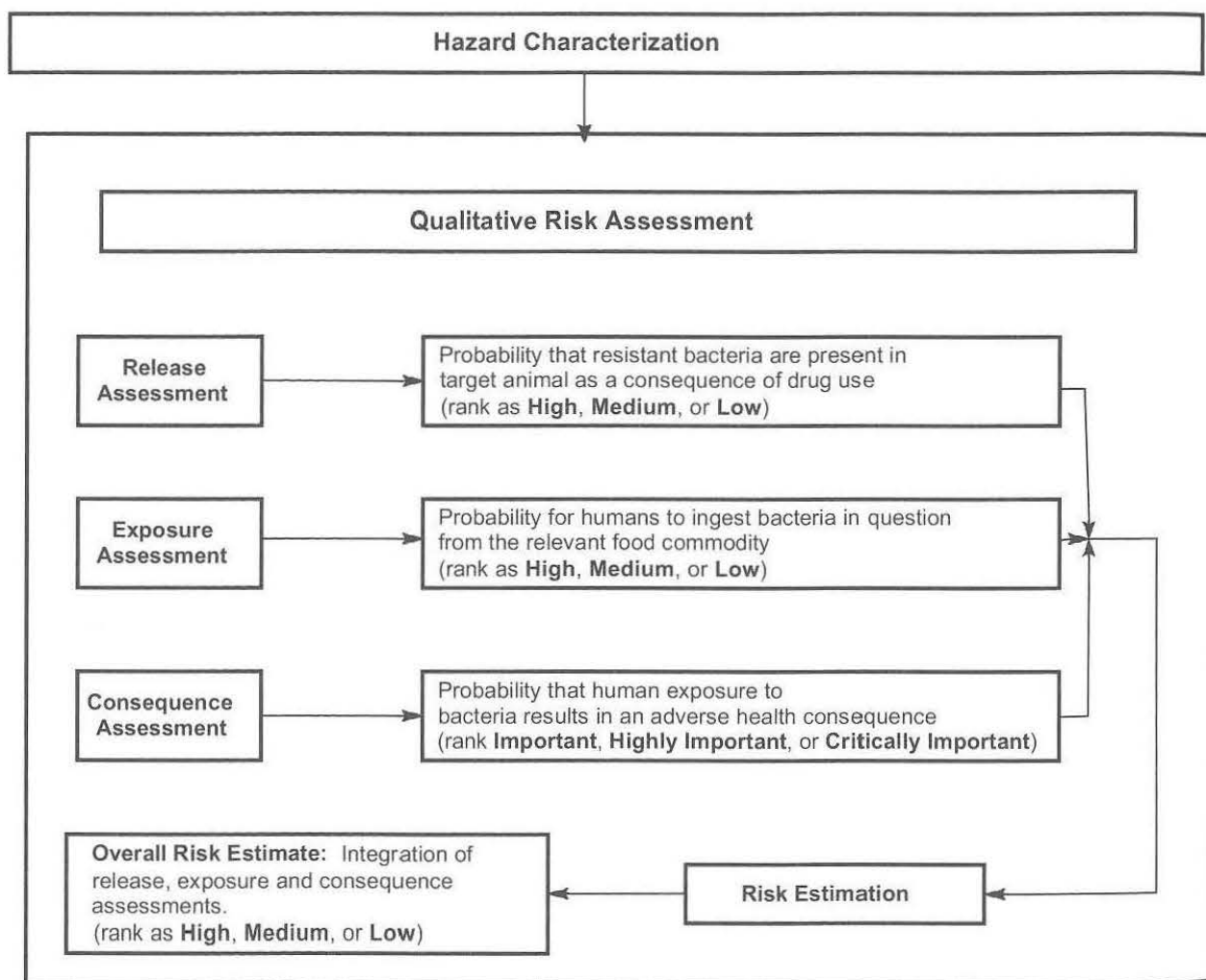


Figure 26.2. US qualitative risk assessment process for evaluation of microbial safety of animal drugs.

Table 26.3. Examples of risk management options in the United States based on the level of risk identified (High, Medium or Low).

Approval conditions	Category 1 (High)	Category 2 (Medium)	Category 3 (Low)
Marketing status*	Rx	Rx/VFD	Rx/VFD/OTC
Extra-label use (ELU)	ELU restrictions	Restricted in some cases	ELU permitted
Extent of use	Low	Low, medium	Low, medium, high
Post-approval monitoring (e.g., NARMS)	Yes	Yes	In certain cases
Advisory committee review considered	Yes	In certain cases	No

*Rx, prescription; VFD, Veterinary Feed Directive; OTC, over-the-counter.

If the qualitative risk assessment shows that the risks are significant, the FDA can deny the application for marketing authorization, thus preventing use of the drug in food animals, or the FDA can approve the drug but place conditions on its use designed to ensure it would not pose a human health risk. Table 26.3 illustrates the risk management options available, stratified by the level of risk. These include prescription or non-prescription status of the antimicrobial; approval in only certain species of animals or only certain routes of administration (e.g., via feed or water versus injectable-only products); and the need for external consulting groups to provide advice.

Different expert bodies in the EU have considered the threat of antimicrobial resistance to both human and animal health. The EU Council passed a resolution in June 1999 on antibiotic resistance titled, "A Strategy against the Microbial Threat". This resolution considered that antibiotic resistance and its various causes require a multidisciplinary and cross-sectorial approach. Also, the European Commission has considered the problem of antimicrobial resistance and delivered the Communication from the European Commission on a community strategy against antimicrobial resistance (EU Commission, 2001). The CVMP carried out a qualitative risk assessment on antimicrobial resistance in the late 1990s (EMA, 1999b) and published a strategic action plan to consider follow-up actions needed in January 2000 (EMA, 1999c). As a result, regulatory guidance for antimicrobials was reviewed and revised guidelines were published (EMA 2001a, 2001b). In the EU, a proposal was made to develop guidance on interpretation of VICH GL27 (2003) data requirements and harmonization of assessment criteria. Before this guidance is available, the EU approach to manage the develop-

ment of antimicrobial resistance is to promote prudent use principles.

Australia, through its Expert Advisory Group on Antimicrobial Resistance, has published draft guidelines for assessing antimicrobial resistance risks that are also based on a qualitative approach (EAGAR).

New Zealand has taken a slightly different approach to the approval of antimicrobial products based on a prudent response in the absence of information on release, exposure, and human health consequences. New Zealand assumes that antibiotic resistance development is possible but uncertain. The New Zealand process attempts to protect the efficacy of essential human health antimicrobial active ingredients by putting safeguards on animal use. This is done while ensuring that information will be available about the use of antimicrobial agents if particular concerns develop or trends in resistance appear. In addition to the basic veterinary prescription condition that establishes a point of control via veterinary involvement, it imposes criteria for use in animals and reporting obligations that are commensurate with existing knowledge of the human health significance of active ingredients and the potential for human exposure. This is balanced with the animal health significance of the active ingredients and the existence or absence of alternative therapeutic choices. The approval authorities for veterinary and human medicines work closely to strike an acceptable compromise that protects both human and animal health as much as possible.

While ingestion is recognized as the most likely pathway, the decision process is equally appropriate for all other pathways. It also provides for pragmatic and prudent regulatory action while relevant information is being generated internationally and in New Zealand to direct more sensitive risk management.

This approach works well for a country as small as New Zealand because almost no antimicrobial products are developed for New Zealand as the primary initial market. Data packages are usually prepared to meet the requirements of other regulatory jurisdictions such as the FDA in the United States or regulatory authorities in the EU. This highlights the value of initiatives such as VICH to harmonize information requirements and risk analysis methodologies.

Prior to 2000, the Agricultural Compounds and Veterinary Medicines Group (ACVM) of the New Zealand Food Safety Authority (NZFSA) followed international regulatory practices and did not assess antibacterial products in regard to the potential to cause antibacterial resistance either in animal pathogens or in contributing to the development of resistance in human pathogens. In 2000 the ACVM Group initiated a review of all currently-approved antimicrobial products relative to antibacterial resistance. The review of antibacterial active ingredients was based on a multi-factorial rationale (Figure 26.3). This rationale considered:

- the relative importance of the active ingredient from a human health perspective
- the likelihood that resistance could develop
- the potential for human exposure via a veterinary use
- the importance of the active ingredient from an animal health and welfare perspective

The review has resulted in a classification of active ingredients relative to their significance to antibacterial resistance (see Table 26.2). The criteria used to classify the antimicrobials in New Zealand differ from those used in the United States. Those drugs of medium to high human health risks with veterinary use require active management of the potential for the development of antibacterial resistance. Those that are of no or low human health significance and for which there are no veterinary use hazards do not require active management from a human health perspective, but may still require management of resistance from an animal health and welfare perspective. The ACVM Group has published its information requirements for veterinary medicine and has also set standards for chemistry, good manufacturing practices, animal safety, efficacy, and residues. These standards are available on the NZFSA/ACVM Group website (www.nzfsa.govt.nz/acvm).

If there are no residual animal health and welfare is-

suces in regard to an active ingredient that is classified as of no concern from a human health perspective, the products could be made available to anyone without a veterinary prescription (i.e., over-the-counter [OTC] sale). If there are residual animal health or welfare concerns or the active ingredient is even of low human health concern, then the veterinary prescription condition is imposed (NZFSA, 2000).

The first level of the stratification (level 1) of the veterinary prescription condition requires the veterinarian to apply sound diagnosis and therapy competencies without specifically drawing attention to the management of antibacterial resistance. This level has been applied to products containing active ingredients of low human health concern.

Products containing active ingredients of medium to high human health importance attract a second level (level 2) stratification, limiting the use to therapeutic purposes under which the prescribing veterinarian is satisfied that the active ingredient is likely to be the only effective treatment. This is allowed because it has been determined that the use of the active ingredient is essential for animal health and welfare.

All products must have label statements to the effect that:

- Indiscriminate use can contribute to the development of antibacterial resistance.
- The product should be used only in individual cases of serious infection that are not likely to respond to any other product.
- The product must not be used to treat groups of food-producing animals unless microbiology has confirmed the diagnosis and susceptibility tests have shown that it is the only alternative that is likely to be effective.

The veterinarian is allowed to exercise discretion to use the product for prophylactic purposes if the potential disease challenge warrants it (i.e., animals have been exposed and are likely to have been infected). Use as a growth promotant is not an approved use. In some cases, the requirement for bacteriology is not practical and alternative management may be used by the prescribing veterinarian to achieve a level of confidence that the use of the product is essential.

Where it is considered essential to control the overall use of an antibacterial active ingredient even for therapeutic purposes, additional controls (level 3

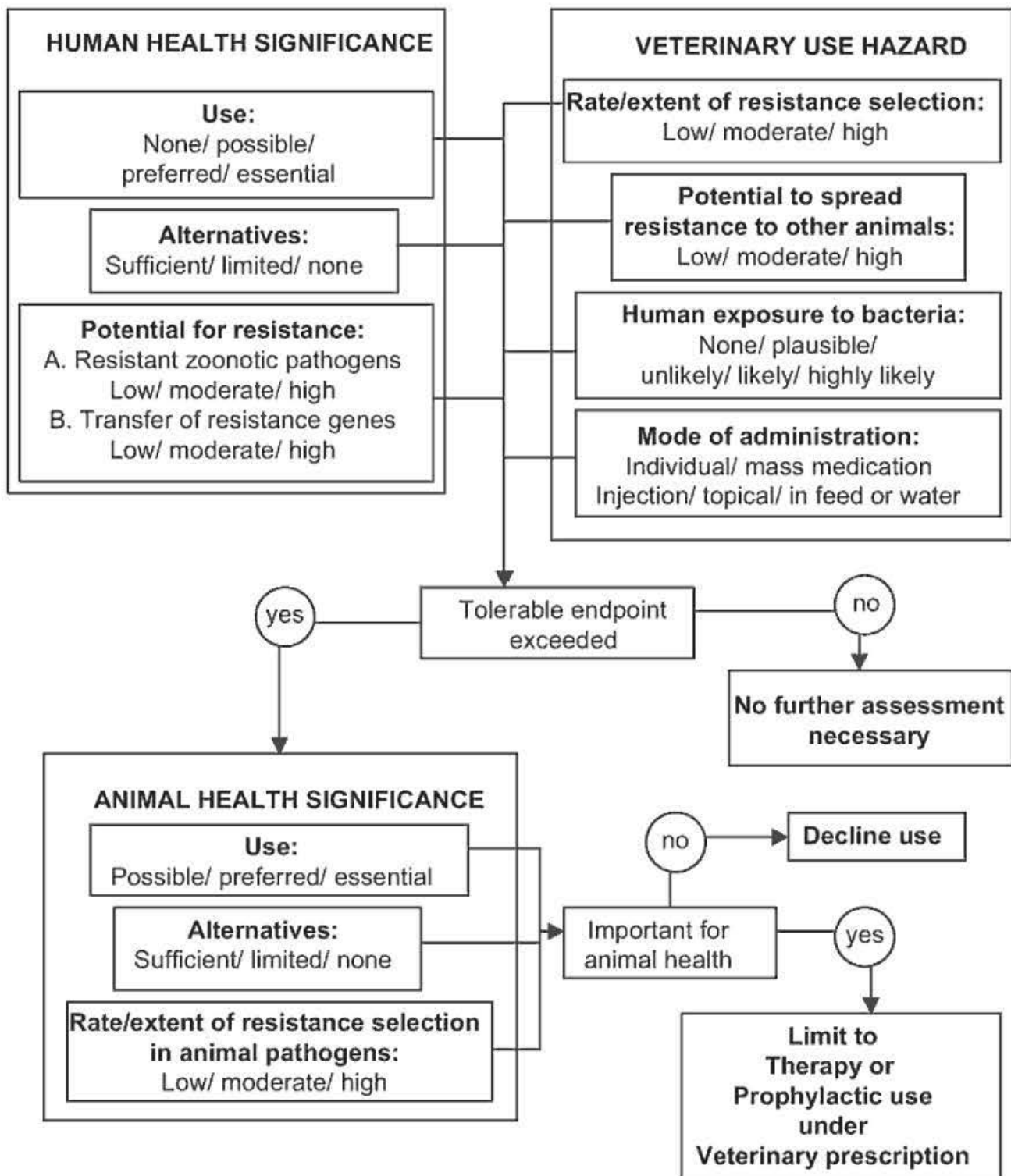


Figure 26.3. Antimicrobial resistance review rationale used in New Zealand.

stratification) are imposed to manage those uses. This level of control would be equivalent to that applied to similar active ingredients that attract more stringent prescribing control in human medicine. Their use would attract the same controls as in the second level of stratification, plus these additional controls:

- All discretionary use for any purpose other than that specified on the label would be prohibited.
- The prescribing veterinarian must notify the ACVM Group of every case in which the product is prescribed, giving the date, species of animal, and disease treated.

Post-Marketing Surveillance

Surveillance for antimicrobial resistance in bacteria from animals, food, and other sources such as animal feed is important in order to:

- follow trends in existing resistance in bacterial strains (or establish baseline resistance levels in those strains)
- detect newly emerging resistance in bacterial strains and direct appropriate research into those areas
- optimize the choice of antimicrobials used in therapy
- monitor the effect of interventions
- provide data for risk analyses to determine risk to human and animal health
- inform policy decisions

OIE reviewed the methods appropriate for surveillance of antimicrobial resistance in bacteria from foods and food animals, focusing on international harmonization (Franklin et al., 2001). OIE also published detailed information on the standardization and harmonization of laboratory methodologies for the detection and quantification of antimicrobial resistance (White et al., 2001).

The WHO convened a consultation on the monitoring of antimicrobial usage in food animals in Oslo, Norway, September 10-13, 2001 (WHO 2001). The purpose of the consultation was to review existing national and international systems for the monitoring of antimicrobial usage in food animals, suggest methods or models that would be useful, and develop recommendations to support the establishment of national monitoring systems for usage of all antimicrobials in food animals. Several recommendations arose from the consultation, including:

- Countries should establish national monitoring programs for the usage of antimicrobial agents in food animals.
- WHO, in collaboration with other relevant international organizations, should recommend a system to identify and classify antimicrobial agents and quantify their use in order to make data comparable.
- Countries should link antimicrobial usage and resistance data.

Thus, the World Health Organization, the World Organization for Animal Health, and the Food and Agriculture Organization of the United Nations have all recommended that countries should implement a monitoring program on both the occurrence of antimicrobial resistance in bacteria from animals and from food of animal origin and on the usage of antimicrobials in animals, and that both systems should be under the purview of the country's competent regulatory authority (WHO, 2000; WHO, 2001; WHO, 2004a; Nicholls et al., 2001).

Several European countries have set up national surveillance programs for drug usage and resistance, including Denmark (DANMAP, 2003), Norway (NORM/NORM-VET, 2004), Finland (FINRES-Vet, 2003), Sweden (SVARM, 2004), and the Netherlands (MARAN, 2002). Surveillance programs exist also in France, Spain and the United Kingdom. In order to harmonize monitoring and reporting on *Salmonella* and *Campylobacter*, the EU in 2003 obligated the member states to monitor antimicrobial resistance in those zoonotic organisms (EU, 2003b). In order to obtain comparable resistance data from the various EU member states, a concerted action has been implemented in a network of 19 veterinary reference laboratories in 18 European countries to harmonize the susceptibility testing of bacteria from food-producing animals. Comparable results are provided to the public and decision-makers.

In the United States, antimicrobial resistance among enteric zoonotic pathogens is monitored nationally in isolates from humans, food-producing animals at federally-inspected slaughter plants, and retail meat in the National Antimicrobial Resistance Monitoring System (NARMS, http://www.fda.gov/cvm/narms_pg.html). Currently, there is no national system for monitoring antimicrobial use in either animals or humans in the US.

In New Zealand, the ACVM Group has monitored

the sale of antibacterial active ingredients in veterinary medicines since 2000. The statistics have been made public in the regulatory control of antibiotics to manage antibiotic resistance progress reports. The sales statistics are considered relatively accurate in regard to total kilograms sold and total kilograms sold for use in feed in specific animal groups such as poultry and pigs. This is because the products are formulated for a particular use in a particular animal group and there are accurate records that can correlate sales with incorporation into feeds formulated for those animal groups. The statistics are not considered particularly accurate in regard to the amount of parenterally administered products in particular animal groups. This is because the products are registered for use in a range of animals to be used against a range of bacterial pathogens. The product registrants who supply the data are asked to estimate amounts likely to have been used for different purposes, which is inherently imprecise.

Conclusion

Antimicrobial veterinary medicinal products provide special challenges both for drug sponsors and regulatory authorities. Antimicrobial resistance not only endangers the efficacy of products in the treatment of animal diseases but can also cause problems in human health. Drug residues can disrupt the human gastrointestinal flora or increase the population of resistant bacteria. Transfer of resistant zoonotic or commensal bacteria or transfer of resistance determinants, either directly from treated animals to humans or indirectly via food, is of growing concern. Many changes in regulatory requirements have taken place during the last few years in an attempt to address the issue of antimicrobial resistance, and it is probable that more changes will occur once more experience has been gained.

Regulators in countries around the world have developed standards for assessing the risks associated with the use of veterinary medicinal products, appropriate market approval requirements, and post-marketing control and surveillance requirements in order to ensure that veterinary antimicrobials are used in a prudent manner. The goal of these efforts, with respect to the issue of antimicrobial resistance, is to balance the need to minimize the impact on human health while having appropriate veterinary medicinal

products available to meet the health and welfare needs of animals.

Bibliography

- Cerniglia CE, Kotarski S. 1999. Evaluation of veterinary drug residues in food for their potential to affect human intestinal microflora. *Regul Toxicol Pharmacol* 29:238.
- Council of the European Union. 1999. Council Resolution of 8 June 1999 on antibiotic resistance: a strategy against the microbial threat. 1999/C 195/01. http://europa.eu.int/eurlex/pri/en/oj/dat/1999/c_195/c_19519990713en00010003.pdf.
- DANMAP. 2003. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food, animals and humans in Denmark. Copenhagen, Denmark: Danish Zoonosis Centre. http://www.dfvt.dk/Files/Filer/Zoonosecentret/Publikationer/Danmap/Danmap_2003.pdf.
- European Medicines Agency (EMA). 1995. Note for guidance: approach towards harmonisation of withdrawal periods for meat. EMA/CVMP/036/95. <http://www.ema.eu.int/pdfs/vet/swp/003695en.pdf>.
- EMA. 1998. Determination of withdrawal periods of milk. EMA/CVMP/473/98. <http://www.ema.eu.int/pdfs/vet/swp/047398en.pdf>.
- EMA. 1999a. Guidelines for the conduct of efficacy studies for intramammary products for use in cattle. EMA/CVMP/344/99. <http://www.ema.eu.int/pdfs/vet/ewp/034499en.pdf>.
- EMA. 1999b. Antibiotic resistance in the European Union associated with therapeutic use of veterinary medicines: report and qualitative risk assessment by the Committee for Veterinary Medical Products. EMA/CVMP/342/99. <http://www.ema.eu.int/pdfs/vet/regaffair/034299ENC.pdf>.
- EMA. 1999c. A risk management strategic plan for controlling antimicrobial resistance through the authorization of veterinary medicine. EMA/CVMP/818/99. <http://www.ema.eu.int/pdfs/vet/regaffair/081899en.pdf>.
- EMA. 2001a. Guideline on antimicrobials for general veterinary use in target animal species. EMA/CVMP/627/01. <http://www.ema.eu.int/pdfs/vet/ewp/062701en.pdf>.
- EMA. 2001b. Guideline on the SPC for antimicrobial products. EMA/CVMP/612/01. <http://www.ema.eu.int/pdfs/vet/ewp/061201en.pdf>.
- EMA. 2005. Guideline on user safety for pharmaceutical veterinary medicinal products. EMA/CVMP/543/03. <http://www.ema.eu.int/pdfs/vet/swp/054303en.pdf>.
- European Parliament. 2003a. Regulation (EC) No 1831/2003 of the European Parliament and the Council on Additives for use in animal nutrition. Official Journal L 268, 18.10.2003, 29-43. <http://europa.eu.int/eur-lex/en/search/index.html>.
- European Parliament. 2003b. Directive 2003/99/EC of the European Parliament and the Council on the monitoring of zoonoses and zoonotic agents, amending Council

- Decision 90/424/EEC and repealing Council Directive 92/117/EEC. Official Journal L 325, 12.12.2003, 31-40. <http://europa.eu.int/eur-lex/en/search/index.html>.
- European Union Commission. 1997. Commission Directive 97/6/EC amending Council Directive 70/524/EEC concerning additives in feedingstuffs. Official Journal L 35, 5.2.1997. <http://europa.eu.int/eur-lex/en/search/index.html>.
- EU Commission. 2001. Communication from the European Commission on a community strategy against antimicrobial resistance. http://europa.eu.int/comm/health/ph/others/antimicrob_resist/am_02_en.pdf.
- EUDRALEX Volume 5. 2005. Pharmaceutical legislation: veterinary medicinal products. <http://pharmacos.eudra.org/F2/eudralex/vol-5/home.htm>.
- EUDRALEX Volume 7AE2a. 1994. Evaluation of the safety of veterinary medicinal products for the target animals. <http://pharmacos.eudra.org/F2/eudralex/vol-7/A/7AE2a.pdf>.
- EUDRALEX Volume 8. 2005. Notice of applicants and note for guidance: Establishment of maximum residue limits (MRLs) for residues of veterinary medicinal products in foodstuffs of animal origin. <http://pharmacos.eudra.org/F2/eudralex/vol-8/home.htm>.
- EUDRALEX Volume 9. 2004. Pharmacovigilance. <http://pharmacos.eudra.org/F2/eudralex/vol-9/home.htm>.
- Expert Advisory Group on Antimicrobial Resistance (EAGAR). Draft framework on risk assessment with respect to applications referred by the national registration authority for agricultural and veterinary chemicals. The National Health and Medical Research Council, Australia. http://www.nhmrc.gov.au/publications/_files/framework.pdf.
- FINRES-Vet. 2003. Finnish veterinary antimicrobial resistance monitoring and consumption of antimicrobial agents. ISSN 1458-6878. Helsinki, Finland: National Veterinary and Food Research Institute (EELA). http://www.eela.fi/linked/fi/julkaisut/finres_vet.pdf.
- Franklin A, et al. 2001. Antimicrobial resistance: harmonization of national antimicrobial resistance monitoring and surveillance programmes in animals and in animal-derived food. *Rev Sci Tech* 20:859.
- JETACAR. 1999. The use of antibiotics in food-producing animals: antibiotic-resistant bacteria in animals and humans. Report of the Joint Expert Advisory Committee on Antibiotic Resistance. Woden, ACT, AUS: Department of Health and Ageing, Commonwealth of Australia. ISBN 1 86496 061 2. <http://www.health.gov.au/internet/wcms/publishing.nsf/Content/health-pubs-jetacar-cnt.htm>.
- MARAN. 2002. Monitoring of antimicrobial resistance and antibiotic usage in animals in The Netherlands in 2002. Lelystad, The Netherlands: Central Institute for Animal Disease Control. <http://www.cidc-lelystad.wur.nl/UK/publications/>.
- Miller MA, Eirkson CE. 1997. Environmental assessments for animal drug products. Proceedings of the 7th European Association for Veterinary Pharmacology and Toxicology International Congress. *J Vet Pharmacol Therap* 20:323.
- Miller MA, Flynn WT. 2000. Regulation of antibiotic use in animals. In: Prescott, JF, et al., eds. *Antimicrobial Therapy in Veterinary Medicine*, 3rd Edition. Ames, Iowa: Blackwell Publishing. 760-771.
- New Zealand Food Safety Authority. 2000. Guidelines for risk assessment and hazard analysis under the Agricultural Compounds and Veterinary Medicines Act of 1997. <http://www.nzfsa.govt.nz>.
- Nicholls T, et al. 2001. Antimicrobial resistance: monitoring the quantities of antimicrobials used in animal husbandry. *Rev Sci Tech Off Int Epiz* 20:841.
- NORM/NORM-VET. 2004. Usage of antimicrobial agents and occurrence of antimicrobial resistance in Norway. ISSN-1502-2307. Tromsø/Oslo, Norway: Norwegian Institute of Public Health. http://www.vetinst.no/Arkiv/Zoonosesenteret/NORM_NORM-VET_2004.pdf.
- Office International des Epizooties. 2001. 2nd OIE Conference on Antimicrobial Resistance: strengthening dialogue between medical doctors and veterinarians. Paris: World Organization for Animal Health (OIE). www.oie.int/eng/press/a_051001.htm.
- SVARM. 2004. Swedish veterinary antimicrobial resistance monitoring. ISSN-1650-6332. Uppsala, Sweden: National Veterinary Institute. <http://www.sva.se/pdf/svarm2004.pdf>.
- Tollefson L, Miller MA. 2000. Antibiotic use in food animals: controlling the human health impact. *J AOAC Int* 83:245.
- US Food and Drug Administration (FDA) Center for Veterinary Medicine. 1989. Guideline 33. Target animal safety guidelines for new animal drugs. <http://www.fda.gov/cvm/Guidance/Guideline33.htm>.
- US FDA Center for Veterinary Medicine. 2003. Guidance for Industry #152. Evaluating the safety of antimicrobial new animal drugs with regard to their microbiological effects on bacteria of human health concern. <http://www.fda.gov/cvm/Guidance/fguide152.pdf>.
- International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH). 2001. Harmonized tripartite guideline GL6. Environmental impact assessments (EIAs) for veterinary medicinal products (VMPs): phase 1. <http://vich.eudra.org/htm/guidelines.htm>.
- VICH. 2001. Harmonized tripartite guideline GL9. Good clinical practice. <http://vich.eudra.org/htm/guidelines.htm>.
- VICH. 2001. Harmonized tripartite guideline GL22. Studies to evaluate the safety of residues of veterinary drugs in human food: reproduction toxicity testing. <http://vich.eudra.org/htm/guidelines.htm>.
- VICH. 2001. Harmonized tripartite guideline GL23. Studies to evaluate the safety of residues of veterinary drugs in human food: genotoxicity testing. <http://vich.eudra.org/htm/guidelines.htm>.
- VICH. 2002. Harmonized tripartite guideline GL28. Studies to evaluate the safety of residues of veterinary drugs in human food: carcinogenicity testing. <http://vich.eudra.org/htm/guidelines.htm>.
- VICH. 2002. Harmonized tripartite guideline GL31. Studies to evaluate the safety of residues of veterinary drugs in

- human food: repeat-dose (90-day) toxicity testing. <http://vich.eudra.org/htm/guidelines.htm>.
- VICH. 2002. Harmonized tripartite guideline GL32. Studies to evaluate the safety of residues of veterinary drugs in human food: developmental toxicity testing. <http://vich.eudra.org/htm/guidelines.htm>.
- VICH. 2003. Harmonized tripartite guideline GL27. Guidance on pre-approval information for registration of new veterinary medicinal products for food producing animals with respect to antimicrobial resistance. <http://vich.eudra.org/htm/guidelines.htm>.
- VICH. 2003. Harmonized tripartite guideline GL38. Draft environmental impact assessments (EIAs) for veterinary medicinal products (VMPs): phase II. <http://vich.eudra.org/htm/guidelines.htm>.
- VICH. 2004. Harmonized tripartite guideline GL33. Studies to evaluate the safety of residues of veterinary drugs in human food: general approach to testing. <http://vich.eudra.org/htm/guidelines.htm>.
- VICH. 2004. Harmonized tripartite guideline GL36. Studies to evaluate the safety of residues of veterinary drugs in human food: general approach to establish a microbiological ADI. <http://vich.eudra.org/htm/guidelines.htm>.
- VICH. 2004. Harmonized tripartite guideline GL37. Studies to evaluate the safety of residues of veterinary drugs in human food: repeat-dose (chronic) toxicity testing. <http://vich.eudra.org/htm/guidelines.htm>.
- White DG, et al. 2001. Antimicrobial resistance: standardization and harmonization of laboratory methodologies for the detection and quantification of antimicrobial resistance. *Rev Sci Tech Off Int Epiz.* 20:849.
- World Health Organization (WHO). 1997. The medical impact of the use of antimicrobials in food animals: report and proceedings of a WHO meeting. Berlin, Germany: WHO/EMC/ZOO/97.4.
- WHO. 1998. Use of quinolones in food animals and potential impact on human health: report and proceedings of a WHO meeting. Geneva, Switzerland: WHO/EMC/ZDI/98.12.
- WHO. 2000. Global principles for the containment of antimicrobial resistance in animals intended for food: report of a WHO consultation. Geneva, Switzerland: WHO/CDS//CSR/APH/2000.4.
- WHO. 2001. Monitoring antimicrobial usage in food animals for the protection of human health: report of a WHO consultation. Oslo, Norway: WHO/CDS/CRS/EPH/2002.11.
- WHO. 2004a. Joint FAO/OIE/WHO expert workshop on non-human antimicrobial usage and antimicrobial resistance: scientific assessment. Geneva, Switzerland: WHO/CDS/CPE/ZFK/2004.7. <http://www.who.int/foodsafety/micro/meetings>.
- WHO. 2004b. Second joint FAO/OIE/WHO expert workshop on non-human antimicrobial usage and antimicrobial resistance: management options. Oslo, Norway: WHO/CDS/CPE/ZFK/2004.8.

Prudent Use of Antimicrobials

J. Scott Weese

The advent and subsequent widespread availability of antimicrobial agents was arguably one of the most important milestones in modern medicine. Unfortunately, it was not long until bacteria with decreased susceptibility to antimicrobials were identified. Initially, concerns about antimicrobial resistance were largely tempered with continued development of new drugs. While development of new antimicrobials and antimicrobial classes outpaced development of resistance in the 1950s to early 1970s, the trend has stabilized, if not reversed, and emergence and dissemination of resistant bacteria is a problem worldwide. Antimicrobial resistance can be considered one of the major threats in medicine, and is associated with increased morbidity, mortality, and treatment costs (Shlaes et al., 1997).

Based on the increasing prevalence of antimicrobial resistance and the negative effect on patient outcomes, it is not surprising that factors promoting the development and spread of resistance have come under increasing scrutiny. Antimicrobial misuse and overuse are common in human medicine, where as much as 50% of antimicrobial use may be inappropriate (Gonzales et al., 2001; Lemmen et al., 2001; Yu et al., 1991; Erbay et al., 2005; Paskovaty et al., 2005). Although there is less information for veterinary medicine, inappropriate use of antimicrobials is also a concern and there is increased focus on veterinary antimicrobial use as a source of antimicrobial resistance for humans.

Although any use of antimicrobials can potentially contribute to development of antimicrobial resistance, antimicrobials are a required component of veterinary medicine to improve animal welfare, prevent disease, increase the safety of food, and to treat companion animals, who are important socially and emotionally.

From these standpoints, veterinarians have an ethical responsibility to use antimicrobials when indicated. However, veterinarians also have a responsibility to ensure the wise use of antimicrobials. Accordingly, the focus must be on the appropriate or 'prudent' use of antimicrobials to provide a balance between concerns regarding antimicrobial resistance and positive effects of antimicrobial use.

In recent years, considerable effort has been made to reduce unnecessary use of antimicrobials in human medicine. Although effects are often difficult to accurately quantify, some of these campaigns have led to reduction of resistance in human pathogens such as *Streptococcus pneumoniae* (Low, 2005). Spurred on by the crisis of resistance in human medicine, many national veterinary organizations have developed prudent use guidelines. In addition, many practice-specific veterinary organizations have refined and developed prudent use guidelines.

What is 'Prudent' Use of Antimicrobials?

In broadest terms, prudent use of antimicrobials (also referred to as judicious use or antimicrobial stewardship) is the optimal selection of drug, dose and duration of antimicrobial treatment along with reduction of inappropriate and excessive use, as a means of slowing the emergence of antimicrobial resistance (Shlaes et al., 1997). Specific details defining prudent use are not widely accepted, although many general principles can be applied. The Society for Healthcare Epidemiology in America (SHEA) has published a comprehensive position paper on guidelines for prevention of antimicrobial resistance in human hospitals (Shlaes et al., 1997), key points of which are listed in Table 27.1.

Table 27.1. Society for Healthcare Epidemiology of America recommendations for human hospitals.

Establish a system for monitoring bacterial resistance and usage.
Establish practice guidelines and other policies to control use of antimicrobials, and respond to data from the monitoring system.
Adopt Centers for Disease Control and Prevention (CDC) recommendations for isolation of patients colonized or infected with resistant microorganisms.
Use hospital committees to develop local policies and evaluate policies from other bodies.
Recognize that the financial well-being of the hospital and health of its patients are at stake and that the hospital administration should be accountable for the implementation and enforcement of policies.
Evaluate the effectiveness of policies by measuring outcomes.

Source: Shlaes et al., 1997.

There is a need for veterinary medicine to continue to promote and practice prudent use of antimicrobials. This will involve attempting to move beyond concepts and into generally accepted practice standards that are applicable for all types of veterinary practices. Included in this is the need to find ways to measure outcomes of implementation of antimicrobial use practices, promote continuing education in the area, and ensure that guidelines are dynamic and able to change as needed.

Considerations for Facilitating Prudent Use of Antimicrobials

Antimicrobial Use Guidelines

Development of specific antimicrobial use guidelines can be an effective means of education and can foster prudent antimicrobial use. Typical antimicrobial use guidelines assist in directing appropriate antimicrobial therapy through general or specific recommendations regarding the use of certain drugs or drug classes, or for the treatment of specific diseases or syndromes. In theory, antimicrobial use guidelines should reduce excessive or inappropriate use, both in terms of drug selection and dosing regimens.

Antimicrobial use guidelines in human medicine are mainly available for peri-operative prophylaxis or treatment of common conditions. Local guidelines have been shown to improve the appropriateness of peri-operative therapy (Widdison et al., 1993; Brusaferrero et al., 2001; Alerany et al., 2005). Similarly, prac-

tice guidelines were part of a statewide program to promote appropriate antimicrobial use in Wisconsin and Minnesota that resulted in 19.8 and 20.4%, respectively, decreases in antimicrobial prescription by primary care physicians (Belongia et al., 2005). Conversely, hospitals with the fewest controls on antimicrobial prescribing tend to have the greatest frequency of antimicrobial resistance (Conly, 2002).

Development of antimicrobial guidelines should involve the coordinated effort of a variety of individuals and groups. Depending on the facility and type of guidelines being developed, clinicians, microbiologists, epidemiologists, infectious disease specialists, and pharmacists may be involved.

Overall success of guideline implementation is difficult to measure because of the different outcomes that can be considered, including compliance with guidelines, efficacy of therapy and reduction of antimicrobial resistance. A guideline cannot be realistically considered effective unless all aspects are successful. Whereas outcome of therapy is relatively easy to quantify in terms of response of specific pathogens, overall efficacy of therapy can be clouded by changes in rates of antimicrobial-associated complications (for example, increased *Clostridium difficile*-associated diarrhea in people following changes in prescription patterns in hospitals). Furthermore, objective evaluation of antimicrobial resistance pressure is difficult, and development of resistance by pathogens other than the target pathogen (for example, enteric enterococci) is more difficult to evaluate but should be considered. A guideline that decreases resistance in the target pathogen but increases patient morbidity or increases overall resistance among other pathogens can hardly be considered a success, nor can a protocol that results in poor compliance. Guidelines must be flexible, updated frequently, and locally relevant because of differences in factors such as patient risk and antimicrobial resistance patterns.

Achieving compliance with guidelines can be difficult. A study evaluating compliance with peri-operative guidelines in a human hospital reported good compliance with many aspects of the guidelines, but overall compliance of only 28% (van Kasteren et al., 2003). Reasons for lack of compliance include ineffective distribution of guidelines, lack of awareness of guidelines, and organizational or logistic constraints (van Kasteren et al., 2003). Often, achieving compliance is difficult initially because of resistance to

Table 27.2. Electronic resources for prudent use of antimicrobials in veterinary medicine.

Group	Web address
Canadian Veterinary Medical Association	http://canadianveterinarians.net/publications-resources-order.aspx
American College of Veterinary Internal Medicine	http://www.acvim.org/uploadedFiles/Consensus_Statements/Antimicrobial.pdf
American Association of Feline Practitioners	http://www.aafponline.org/resources/practice_guidelines.htm
American Veterinary Medical Association*	http://www.avma.org/scienact/jtua/jtua98.asp
American Animal Hospital Association	https://www.aahanet.org/About_aaha/About_antimicrobials.html

*Links to species-specific guidelines are also provided.

Table 27.3. Common elements of prudent antimicrobial use guidelines.

Antimicrobials should be used only when there is reasonable likelihood that bacterial infection is present or imminent.
Antimicrobial therapy should be based on culture and susceptibility testing whenever possible.
As narrow a spectrum of therapy as possible should be used.
Antimicrobials should be used for as short a time as possible.
Antimicrobial, pathogen, infection site, and patient factors should be considered when choosing an appropriate treatment.
Antimicrobials that are important for treating refractory or serious infections in humans should be used sparingly in animals and only after careful consideration.
Extra-label use should be avoided when on-label options are reasonable.
Clients should be educated to improve compliance, particularly with respect to completing the entire treatment course.
Antimicrobial therapy should never be used as a substitute for good infection control, medical and surgical practices, and animal husbandry.
Methods to reduce the risk and incidence of infection should be emphasized to decrease the need for antimicrobials.
Peri-operative prophylaxis should be used only when indicated, and following standard guidelines.
Antimicrobials should only be used in the context of a valid veterinarian/client/patient relationship.

change, but can improve when efforts to educate and improve compliance persist.

A variety of veterinary groups have developed prudent use guidelines (Table 27.2). Most veterinary guidelines are relatively generic and emphasize a few key important points (Table 27.3). These points should be considered whenever antimicrobials are prescribed. While important, many published guidelines do not provide enough specific information to guide antimicrobial use in general veterinary practice. This may be based on a desire to avoid controversy and concerns about restricting the flexibility of members to make treatment decisions.

Perhaps the most comprehensive approach toward antimicrobial guidelines in companion animals is a consensus statement regarding antimicrobial use in large animals by the American College of Veterinary Internal Medicine (Morley et al., 2005). Specific use recommendations were not made; however, this position statement can be useful as a guide for implementation of local, specific guidelines.

Some facilities, mainly veterinary teaching hospitals, have developed specific antimicrobial use policies, based on general prudent use guidelines but incorporating details regarding the use of antimicrobials in different situations. These are usually developed in response to concerns about use of a specific antimicrobial or use in certain situations. There has been minimal evaluation of the impact of antimicrobial use guidelines in veterinary medicine. In one of the few reported studies, implementation of guidelines was shown to be effective at reducing the overall use of antimicrobials and reducing the use of certain targeted antimicrobials (fluoroquinolones, carbapenems) in small animals (Weese, 2006).

Antimicrobial Use Categories

It has been recommended that veterinarians and veterinary practices categorize antimicrobials used at their facilities as to use (Morley et al., 2005). Use categories define when the use of specific drugs is appropriate. Various category names have been employed, such as primary, secondary and tertiary, or 1st-line, 2nd-line and 3rd-line. There are no recommendations for defining use categories or placing drugs within these categories, but basic principles should remain the same. An example of definition of use categories is presented in Table 27.4.

The key principle of use categorization is that higher class drugs should only be used when lower class drugs are not appropriate, when alternative ap-

Table 27.4. Categorization of antimicrobials.

Class	Definition	Examples
Primary/1st-line	Used as initial treatments with known or suspected bacterial infections, in advance of or in lieu of culture and susceptibility results. These drugs may be commonly used in human medicine but are typically considered less important for treating serious human infections or raise less concern about development of resistance.	Penicillin, most cephalosporins, trimethoprim-sulfonamides, tetracyclines
Secondary/2nd-line	Used when culture and susceptibility testing, plus patient and infection factors, indicate that no 1st-line drugs are reasonable options. Drugs in this class may be more important for treatment of serious infection in humans, or there may be particular concern about development of resistance.	Fluoroquinolones, 3rd and later generation cephalosporins
Tertiary/3rd-line/‘big-gun’	Used in serious, life-threatening infections, with the support of culture and susceptibility testing, when no 1st- or 2nd-line drugs are indicated.	Carbapenems
Restricted, voluntarily prohibited	Used only in life-threatening infections where culture and susceptibility testing indicates no other options. Additional requirements may be indicated, or use may be voluntarily prohibited.	Vancomycin

proaches (i.e., local therapy alone) are not appropriate, and when treatment has a reasonable potential to positively affect the outcome of the case. Culture and susceptibility testing of appropriate diagnostic specimens is essential for determining the use of different classes. The categorization of antimicrobial drugs for animal use based on their importance in human medicine is an area of current intense discussion by medical infectious disease specialists (for example, the World Health Organization, 2005) and animal drug use regulatory authorities (for example, the US Food and Drug Administration Center for Veterinary Medicine, 2003).

Placement of specific drugs into use categories may vary somewhat with practice type and animal species, but the general principles remain the same. It is critical to remember that primary (1st-line) drugs are not necessarily ‘weaker’ or less useful than secondary or tertiary drugs. Seriousness of infection should not by itself indicate the need for 2nd- or 3rd-line drugs. In fact, 1st-line drugs are useful for the treatment of the vast majority of bacterial infections. At one tertiary care small animal teaching hospital, first-line drugs still accounted for over 90% of antimicrobial prescriptions, despite a caseload skewed towards critically ill referral cases, many of which had been treated with a variety of antimicrobials prior to presentation and were immunocompromised (Weese, 2005). Development of practice specific categories, taking into con-

sideration the patient population, antimicrobial resistance patterns and drug factors, can be a useful exercise in promoting prudent use.

Consultation with clinical microbiologists, clinical specialists such as internists, and infection control personnel is very useful and should be undertaken when situations seem to indicate that 2nd- or 3rd-line antimicrobials are indicated, and could reasonably be considered a mandatory component of 3rd-line drug selection.

Antimicrobial Restriction Policies

Antimicrobial restriction is a contentious measure, but formal or informal restriction of the availability of antimicrobials is a well-recognized approach in human medicine. Restriction may involve an outright ban of certain drugs, but more commonly requires demonstration of the need for a specific antimicrobial and authorization by designated officials prior to its use. A 1996 survey reported that 81% of surveyed human teaching hospitals had a policy that restricted use of certain antimicrobials (Lesar and Briceland, 1996). Although beneficial results have been demonstrated in human medicine, implementation of voluntary restriction policies remains controversial and under-investigated in veterinary medicine. Some clinicians may think that antimicrobial restriction causes undue obstruction to their privilege of using off-label therapies, whereas others may believe that restriction

of certain drugs or classes is an appropriate response to concerns regarding antimicrobial misuse and veterinary use of drugs that are deemed critically important in human medicine.

Some veterinary teaching institutions voluntarily restrict the use of certain drugs such as vancomycin. At the Ontario Veterinary College, systemic vancomycin may only be used in cases with life-threatening infection, where local therapy alone is not reasonable, where there is the potential for survival with therapy, when culture and susceptibility testing indicates that vancomycin is the only option, and with the approval of two designated members of the Infection Control Committee. Some other facilities have outright bans on the use of vancomycin, whereas others permit its use without restriction.

All veterinary facilities should consider whether specific antimicrobial restriction policies are indicated. Even in situations where the target drug has never been used, it is reasonable to proactively develop a policy so that decisions can be made in advance, with consultation of relevant parties, as opposed to a reactionary process when a clinician wishes to use the drug. Education and creation of open dialogue between all involved parties is critical to facilitate development and successful implementation of voluntary restriction policies.

Formularies in Promoting Prudent Use

Drug formularies can take two major forms. One type lists antimicrobial drugs that are to be made available for an institution or region. Another type involves treatment recommendations for common conditions, including antimicrobial agent, dose, route and duration. In human medicine, formularies are mostly used to control antimicrobial costs, but they can have a significant impact on antimicrobial use trends. In some areas, most (66% to 91%) human hospitals have a formulary (Lawton et al., 2000; Moro et al., 2003; Woodford et al., 2004), and changes in formulary composition can have a profound impact on the use of certain antimicrobials. Regional formularies can include local data on antimicrobial resistance trends, and regular reviews can ensure that protocols remain adequate. Formularies are not widespread in veterinary medicine, and may be met with resistance based on negatively impacting the 'freedom' of practice and the 'art' of veterinary medicine alluded to earlier. Development of standardized antimicrobial use rec-

ommendations for common situations has been advocated (Morley et al., 2005), with the understanding that the goal is not to dictate the practice of medicine, but rather to supplement clinical judgment and ensure that the art of medicine remains based in science.

Stop Orders in Promoting Prudent Use

A potential cause of excessive antimicrobial use is failure to discontinue therapy at the appropriate time. In some cases, antimicrobial treatment may be continued for longer than planned simply because no one wrote orders to stop therapy. Stop orders are automatic orders to stop antimicrobial therapy after a certain length of time. Longer treatment is permitted but must be requested; therefore inadvertent continuation of therapy is avoided. Stop orders are less applicable in veterinary medicine apart from hospitals where antimicrobials are dispensed from a pharmacy. Important practical applications of this concept include ensuring that animals are re-evaluated before renewal of any antimicrobial prescription and not dispensing bulk quantities of drugs to be used at an owner's or producer's discretion.

Introduction of New Antimicrobials

Veterinary medicine is a progressive medical field, and practitioners typically strive to remain on the cutting edge of medicine. As a consequence, there may be pressure to try new antimicrobials, or drugs that are used in humans but rarely used in animals. The use of newer antimicrobials when traditional options are reasonable does not constitute progress in the practice of medicine but rather may indicate inappropriate use. As antimicrobial resistance develops, there will be increased pressure, and in many cases need, to use newer antimicrobials. The key for prudent use is avoiding these changes until absolutely required, and ensuring that use of these drugs will have as minimal impact on antimicrobial resistance in human and animal pathogens as possible.

One conundrum is at what stage one starts using new antimicrobials. Objective guidelines have not been developed to assist with this process. Consideration should be given to defining situations where first-line empirical use of a drug for a particular pathogen is inappropriate. This is difficult to do without supporting evidence, and is confounded by regional variations in susceptibility, effects beyond antimicrobial spectrum that influence clinical outcome, and the bias

of clinical laboratory surveillance results, whereby worst-case data is usually obtained.

Clinical Microbiology in Prudent Use: The Role of the Diagnostic Laboratory

Submission of appropriate diagnostic specimens is a critical but often neglected aspect of prudent use. Identification of the offending organism may allow for use of a narrow-spectrum antimicrobial, and give added insight into the antimicrobial regimen required. The consequences of failure to collect a diagnostic sample may include initial treatment failure, with potential increases in morbidity, mortality, treatment costs and overall antimicrobial use.

Monitoring resistance trends in important pathogens can be useful in any practice as a guide to appropriate antimicrobial use in advance of susceptibility testing results, or in cases where testing is not performed. However, using data from diagnostic labs can be misleading, since results only indicate the prevalence of resistance in bacteria isolated from clinical specimens submitted to the diagnostic laboratory. This population can be biased towards more severe infections, infections that develop despite prophylactic antimicrobial therapy, and infections that are non-responsive to initial therapy; hence results do not necessarily indicate the overall prevalence of resistance. Resistance in pathogens isolated in the diagnostic laboratory is usually higher than resistance in the same pathogens from untreated animals. If the overall prevalence of resistance is to be determined, surveillance must include specimens not sourced from diagnostic laboratories.

The laboratory can have an impact on antimicrobial use based on reporting of culture and sensitivity testing results. The Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) has standard guidelines for testing and reporting of antimicrobial susceptibility (NCCLS, 2004). These guidelines provide information on what antimicrobials should be tested for certain bacteria and which organisms should be considered resistant to certain antimicrobials regardless of testing results (for example, cephalosporin resistance in enterococci). Reporting of antimicrobial susceptibility results for a large number of drugs, particularly 2nd- and 3rd-line drugs, can result in increased unnecessary use of these agents. It is also important that laboratories test certain bacterial species for resistance patterns that are of particular relevance, such as

oxacillin (methicillin) resistant *Staphylococcus aureus* or vancomycin resistant enterococci. Another important component is not performing susceptibility testing on bacterial isolates that are considered to be part of the normal microflora, and not considered clinically relevant given the site of sampling and clinical process. Reporting of susceptibility results for commensal microflora undoubtedly results in inappropriate antimicrobial use.

It is also important to remember that results are only as good as the methodology used to produce them. Results obtained by laboratories with minimal quality control or without using internationally agreed standards for methodology and susceptibility breakpoints must be interpreted with care (discussed more fully in Chapter 2). Objectively developed standards that are of relevance to all veterinary species are required. The CLSI Veterinary Antimicrobial Susceptibility Testing (VAST) committee has made important advances in this area, but its methods are focused on United States needs and largely on food animal usage. International standardization of methodologies and accreditation of diagnostic laboratories would be an important advance in the area, particularly if it was combined with ongoing electronic data capture and frequent publication of antimicrobial resistance data.

Collection of data regarding antimicrobial resistance patterns is useful to guide proper treatment selection, identify emerging problems, and assist in the objective development of antimicrobial use policies. Proper monitoring requires regular submission of appropriate diagnostic specimens and monitoring of resistance patterns in the commensal microflora. Evaluation of antimicrobial susceptibility patterns can be used as an initial screening technique to identify outbreaks. Specific testing, such as pulsed field gel electrophoresis, can be very useful for characterization of changes in antimicrobial susceptibility trends and evaluation of potential outbreaks.

Diagnostic Testing

It is important that reasonable measures be taken to achieve a diagnosis so that antimicrobials are not used unnecessarily *and* so that antimicrobials are properly used if indicated. A common limitation is the reluctance of owners to pay for diagnostic testing. Although it is unreasonable to expect that all animal owners will agree to recommended testing, the manner in which diagnostic testing is presented can have a significant

impact on compliance. It is important that the benefits of obtaining a prompt and accurate diagnosis are outlined. This can increase compliance with testing, thereby improving medical care overall and potentially decreasing 'shot-gun' antimicrobial therapy in non-specific diseases where a diagnosis is not available.

Development of Practice-based Prudent Use Guidelines

It has been recommended that every practice, regardless of type or size, should have an infection control program of some variety (Morley et al., 2005). Antimicrobial use guidelines should be incorporated into this. This may be as simple as providing a copy of species or specialty-relevant guidelines that have already been prepared (Table 27.2), or including practice-specific descriptions of antimicrobial use classes (first-line, second-line, etc.), guidelines for use of each class, and antimicrobial restriction policies. Unfortunately, availability of some proprietary data is limited, which hampers widespread evaluation and comparison of guidelines from different areas. Because of the cross-species and international importance of antimicrobial resistance, it is important that groups disseminate rather than restrict access to their guidelines. As part of an overall infection control program, veterinary practices should consider factors that might indicate an increased risk of antimicrobial resistance in their facility. While these factors have not been defined for veterinary medicine, extrapolation from human hospitals is reasonable (Table 27.5).

An additional benefit of antimicrobial use guidelines is in providing support to the veterinarian should problems develop with a case. This is particularly true in a situation where antimicrobial therapy is not indicated and not used, and an infectious complication develops. The availability of guidelines supporting the initial treatment decision may be of use should litigation or complaint to a licensing body ensue. This may be most important with peri-operative antimicrobial use (Chapter 21), an area where antimicrobials are undoubtedly overused.

Education in Promoting Prudent Use

Although general awareness of antimicrobial resistance and concerns regarding overuse of antimicrobials is increasing in the lay population, veterinarians may still face pressure from owners or producers to dispense antimicrobials in situations where antimicrobial

Table 27.5. Factors associated with increased antimicrobial resistance potential in veterinary clinics.

Hospital facility versus ambulatory practice.
Greater population of sick, hospitalized patients versus elective inpatients or outpatients.
Greater severity of disease in hospitalized patients.
More severely immunocompromised patients.
Increased introduction of resistance organisms from individuals in the community.
Ineffective (or absent) infection control and isolation practices.
Increased use of antimicrobial prophylaxis.
Increased empirical antimicrobial therapy.
High level of overall antimicrobial use.

Source: Shlaes et al., 1997.

therapy is not indicated. Increased education, both on an individual veterinarian-client level, and from broader initiatives, may reduce antimicrobial use. Education of veterinarians, from students to experienced practitioners, could play an important role in ensuring appropriate use of antimicrobials. Funding for such ventures may require support from governments, since this is a matter of broad societal significance.

A potentially weak link in the antimicrobial use path is clients. The best-designed and implemented prudent use program can be easily compromised by client attitudes and actions. Education of clients is crucial because client pressure to 'do something' or prescribe antimicrobials can lead to inappropriate antimicrobial use. Further, failure to complete the prescribed treatment course is a concern, both in terms of the treated animal and the potential that the remaining drug may be used without veterinary input in the future. Good communication with clients can overcome the pressure to prescribe inappropriately and can emphasize the need to complete the entire treatment course. Additionally, veterinarians must take into consideration the abilities of the owner and the behavior of the animal when prescribing to decrease the likelihood of poor compliance because of difficulty in administering the drug (route or frequency of administration), which can lead to premature cessation of therapy.

Use of Non-antimicrobial Treatment Options

Antimicrobials must be considered only one aspect of the treatment plan. Proper concurrent treatment, in-

cluding animal management, can have a beneficial impact on response to treatment and perhaps decrease the need for longer term or repeated use.

An area gathering increasing attention is the use of non-antimicrobial treatments, as complementary therapy or in place of antimicrobials. Probiotics and immunostimulants or immunomodulators are widely used; however, there is little objective evidence of efficacy (or safety) at this point. Quality control of veterinary probiotics appears to be poor (Weese, 2002, 2003) and probiotic therapy with one organism was recently reported to be associated with development of diarrhea in foals (Weese and Rousseau, 2005). The lack of proper quality control and scientific testing may be a result of classification of most of these products as nutritional supplements rather than pharmaceutical agents. More stringent regulation of complementary therapies and pressure for proper research and testing is needed to determine whether these modalities may be useful for the treatment or prevention of disease and the reduction of antimicrobial use and resistance.

Infection Control in Promoting Prudent Use

The role of infection control in prudent use of antimicrobials cannot be over-emphasized and is an important component in reducing spread of resistant bacteria or their resistance genes. Whereas use of the proper antimicrobial regimens is important, prevention of infection (or more accurately reduction in infection rates) can decrease the need to consider any of these factors. Antimicrobials should never be used as a substitute for good animal husbandry and infection control (Chapter 24). Veterinary assistance with development of policies for good management practices, and infection control programs for farms, veterinary facilities and the household, is an important part of prudent use of antimicrobials. Further, monitoring of infection rates, antimicrobial susceptibility trends, and antimicrobial use patterns can provide important data.

In human medicine, it has been estimated that 30-70% of nosocomial infections are preventable. When one considers the impact of nosocomial infection and the significant amount of antimicrobial use that presumably occurs as a result of nosocomial infection in veterinary hospitals, the potential impact of infection control practices becomes readily apparent. In particular, the role of simple hand hygiene must not be overlooked as a tool to decrease antimicrobial use by preventing spread of infectious agents, including resistant

bacteria. Hand hygiene has been shown to be among the most effective infection control practices, and proper hand hygiene can have significant impacts on infection rates (Hirschmann et al., 2001; Boyce and Pittet, 2002).

Access to Antimicrobials and Implications for Prudent Use

In most countries, the veterinarian is directly responsible for overseeing and directing antimicrobial use in animals. This is obviously a logical approach because of veterinarians' training in animal diseases, animal husbandry and antimicrobial use. However, in many countries some antimicrobial agents remain available for purchase over the counter by lay personnel, with no direct veterinary involvement. As such, it has been recommended that all antimicrobials be available only through a veterinarian with an established veterinarian-patient-client relationship (Morley, 2005). Not only should this reduce resistance, this is one way in which the total antimicrobial drug use in a country could be readily monitored.

Internal versus External Regulation in Promoting Prudent Use

Currently, veterinarians in many countries have almost complete freedom in dispensing any antimicrobial that is available for human use, with the only regulatory issues involving use of certain antimicrobials in food animals and concerns about antimicrobial residues in those species. In some countries, veterinary dispensing of antimicrobials is tightly controlled, and there are continual calls in many countries to restrict veterinary access to antimicrobials. One approach that can be taken to decrease the likelihood of restrictions being placed on antimicrobial use is demonstration of prudent use and self-regulation. Internal 'policing', including guideline development, internal quality control and emphasizing an evidence-based approach to antimicrobial use, can demonstrate that care and attention is being paid to antimicrobial use and resistance issues, and may help allay concerns by non-veterinary groups. To decrease the perceived need for external control, it is critical to demonstrate to regulatory bodies that veterinary medicine is taking a proactive, prudent approach to antimicrobial use. Concerns regarding inappropriate antimicrobial use practices in animals have been raised, sometimes quite vocally, and should serve as a stimulus for the veterinary profession to take measures

to demonstrate prudent use. Failure to do so could increase the likelihood of severe restrictions being placed on veterinary access to antimicrobials.

Importance of Regulatory Monitoring for Evaluation of Prudent Use Measures

A variety of regulatory agencies are involved internationally in evaluation of risks with new antimicrobials, licensing of veterinary antimicrobials and post-licensing surveillance for resistance (Chapter 26). Monitoring programs should include evaluation of resistance in both target organisms (the organisms against which antimicrobial therapy is directed) and indicator bacteria (organisms not the target of therapy but in which antimicrobial resistance is of relevance). For example, evaluation of a new drug class in cattle for treatment of bovine respiratory disease complex should be accompanied by surveillance of resistance among organisms such as commensal *E. coli*, not just *Mannheimia* and *Pasteurella*. Development of resistance in either group is of concern, and in some cases resistance in indicator bacteria may be more important because they can be important zoonotic pathogens. Both groups must be considered in post-licensing surveillance and ongoing antimicrobial resistance screening. Regulatory bodies may withdraw access to the antimicrobial if concerning trends develop in either group; however, this is most likely to occur if resistance in human pathogens develops. In the United States, the Food and Drug Administration (FDA) Center for Veterinary Medicine recently withdrew approval for the use of enrofloxacin in poultry because of emergence of ciprofloxacin resistance in *Campylobacter jejuni* (Chapter 3).

Only a few countries have embarked on programs to aggressively reduce the use of antimicrobials in animals; however, the pressure to do so may increase. One of the most comprehensive is the Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP), which reports antimicrobial use data from animals and humans, as well as the occurrence of antimicrobial resistance in bacteria from animals, humans, and food products (DANMAP, 2004). Concurrent monitoring of antimicrobial use and resistance patterns can assist in development of prudent use guidelines; however, there are limitations to the quality of data generated. Some findings of the DANMAP program are not surprising, such as increased resistance of *E. coli* and *Salmonella typhi-*

murium to tetracycline and ampicillin coinciding with increased consumption of tetracycline and broad-spectrum penicillins in pigs, and a greater prevalence of antimicrobial resistance in *S. typhimurium* and *Enterococcus* spp. isolates in pork imported from countries without antimicrobial restrictions comparable to those in Denmark. However, some findings are not as easy to explain and show that antimicrobial resistance trends are not necessarily easy to predict. For example, fluoroquinolone resistance in *Campylobacter* isolates from pigs increased while fluoroquinolone use decreased. Similarly, erythromycin resistance in *Campylobacter coli* did not increase despite a substantial reported increase in macrolide use in weaned pigs during the same period, but resistance in *Enterococcus faecium* did increase.

A major component of the Danish program has been a ban on the use of antimicrobials for growth production. The effects of this program on total antimicrobial consumption in animals were striking: Total animal consumption of antimicrobials decreased from 169 metric tons in 1997 to 80 metric tons in 1999. However, there has been a steady increase in therapeutic use in animals since then (DANMAP 2004) (Chapter 24).

A weakness of most monitoring programs is the focus on food animals. The more frequent use of drugs that the FDA classifies as critically important (i.e., fluoroquinolones and cephalosporins) and the close nature of contact between humans and companion animals indicates the potential importance of companion animals in the emergence of antimicrobial resistance (Heuer et al., 2005). Assessment of prudent use by focusing solely on the effects of food animal antimicrobial use on human pathogens may provide only part of the picture.

Vaccination and other Measures as a Means to Promote Prudent Use

The potential role of vaccination in prudent use of antimicrobials should not be overlooked. Whereas vaccination does not have a direct effect on resistant bacterial populations, decreasing the incidence of disease in animals can reduce the need for and use of antimicrobials. Introduction of a pneumococcal vaccine in humans quickly resulted in a decrease in antimicrobial use and antimicrobial resistance in *Streptococcus pneumoniae* (Dagan et al., 2001; Talbot et al., 2004). This does not apply only to bacterial diseases. Vaccination

against viral disease can reduce the incidence of secondary bacterial disease that might require antimicrobial treatment and decrease potential (inappropriate) antimicrobial use in primary viral disease.

Conclusion

Antimicrobial resistance will not disappear and will continue to have a marked impact on the practice of human and veterinary medicine. Prudent use of antimicrobials is critical to reduce the incidence of antimicrobial resistance and decrease the emergence of new resistance genotypes and phenotypes, as well as to reduce the wide variety of genetic elements that capture and move resistance genes in bacterial populations. Prudent use ensures veterinarians continued access to required antimicrobials. It maximizes the benefits of antimicrobial therapy both for individual patients and from a public health standpoint. Defining and achieving prudent but effective use of antimicrobials is one of the major challenges facing veterinary and human medicine.

Bibliography

- Alerany C, et al. 2005. Impact of local guidelines and an integrated dispensing system on antibiotic prophylaxis quality in a surgery centre. *J Hosp Infect* 60:111.
- Belongia EA, et al. 2005. Impact of statewide program to promote appropriate antimicrobial drug use. *Emerg Infect Dis* 11:912.
- Boyce JM, Pittet D. 2002. Guideline for hand hygiene in health-care settings: recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA hand hygiene task force. *Infect Control Hosp Epidemiol* 23 (Suppl):S3.
- Brusaferrro S, et al. 2001. Protocol implementation in hospital infection control practice: an Italian experience of pre-operative antibiotic prophylaxis. *J Hosp Infect* 47:288.
- Conly J. 2002. Antimicrobial resistance in Canada. *Can Med Assoc J* 167:885.
- Dagan R, et al. 2001. Effect of a conjugate pneumococcal vaccine on the occurrence of respiratory infections and antibiotic use in day-care center attendees. *Pediatr Infect Dis J* 20:951.
- DANMAP. 2004. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. Copenhagen, Denmark: Danish Zoonosis Centre.
- Erbay A, et al. 2005. Evaluation of antibiotic use in intensive care units of a tertiary care hospital in Turkey. *J Hosp Infect* 59:53.
- Gonzales R, et al. 2001. Excessive antibiotic use for acute respiratory infections in the United States. *Clin Infect Dis* 33:757.
- Heuer OE, et al. 2005. Antimicrobial drug consumption in companion animals. *Emerg Infect Dis* 11:344.
- Hirschmann H, et al. 2001. The influence of hand hygiene protocols prior to insertion of peripheral venous catheters on the frequency of complications. *J Hosp Infect* 49:199.
- Lawton RM, et al. 2000. Practices to improve antimicrobial use at 47 US hospitals: the status of the 1997 SHEA/IDSA position paper recommendations. Society for Healthcare Epidemiology of America/Infectious Diseases Society of America. *Infect Control Hosp Epidemiol* 21:256.
- Lemmen SW, et al. 2001. Influence of an infectious disease consulting service on quality and costs of antibiotic prescriptions in a university hospital. *Scand J Infect Dis* 33:219.
- Lesar TS, Briceland LL. 1996. Survey of antibiotic control policies in university-affiliated teaching institutions. *Ann Pharmacother* 30:31.
- Low DE. 2005. Changing trends in antimicrobial-resistant pneumococci: it's not all bad news. *Clin Infect Dis* 41 Suppl 4, S228.
- Morley PS, et al. 2005. Antimicrobial drug use in veterinary medicine. *J Vet Intern Med* 19:617.
- Moro ML, et al. 2003. Antibiotic policies in Italian hospitals: still a lot to achieve. *Microb Drug Resist* 9:219.
- National Committee for Clinical Laboratory Standards. 2004. Fourteenth Informational Supplement M100-S14. Wayne, PA: Clinical and Laboratory Standards Institute.
- Paskovaty A, et al. 2005. A multidisciplinary approach to antimicrobial stewardship: evolution into the 21st century. *Int J Antimicrob Agents* 25:1.
- Shlaes DM, et al. 1997. Society for Healthcare Epidemiology of America and Infectious Diseases Society of America Joint Committee on the Prevention of Antimicrobial Resistance: Guidelines for the prevention of antimicrobial resistance in hospitals. *Infect Control Hosp Epidemiol* 18:275.
- Talbot TR, et al. 2004. Reduction in high rates of antibiotic-nonsusceptible invasive pneumococcal disease in Tennessee after introduction of the pneumococcal conjugate vaccine. *Clin Infect Dis* 39: 641.
- Van Kasteren ME, et al. 2003. Adherence to local hospital guidelines for surgical antimicrobial prophylaxis: a multi-centre audit in Dutch hospitals. *J Antimicrob Chemother* 51: 1389.
- Weese JS. 2002. Microbiologic evaluation of commercial probiotics. *J Am Vet Med Assoc* 220:794.
- Weese JS. 2003. Evaluation of deficiencies in labeling of commercial probiotics. *Can Vet J* 44:982.
- Weese JS. 2006. Antimicrobial use in a small animal veterinary teaching hospital and the impact of antimicrobial use guideline. *J Am Vet Med Assoc* 228:553.
- Widdison AL, et al. 1993. Survey of guidelines for antimicrobial prophylaxis in surgery. *J Hosp Infect* 25:199.
- Woodford EM, et al. 2004. Documentation of antibiotic prescribing controls in UK NHS hospitals. *J Antimicrob Chemother* 53:650.
- Yu V L, et al. 1991. Empiric antibiotic selection by physicians: evaluation of reasoning strategies. *Am J Med Sci* 301: 165.

Section IV

Antimicrobial Drug Use in Selected Animal Species

Antimicrobial Drug Use in Horses

Steeve Giguère

Rational drug therapy is defined as the selection of the proper drug to be administered according to a dosage regimen appropriate to the patient after due appraisal of potential therapeutic benefits and risks. The first step in this decision-making process is to determine whether an infectious agent is the likely cause of the disease, and if so, that the animal is unlikely to efficiently eliminate the infection without antibiotic therapy. In choosing the appropriate antimicrobial agent, the veterinarian must consider: (1) the likely identity of the infecting microorganism(s); (2) its in vitro antimicrobial susceptibility pattern or the clinical response in equine patients infected with the same pathogen; (3) the nature and site of the infectious disease process; (4) the pharmacokinetic characteristics of the chosen antimicrobial agent in horses, such as bioavailability, tissue distribution, and rate of elimination; (5) the pharmacodynamic properties of the antimicrobial agent selected; (6) its safety in horses; and (7) the cost of therapy.

Because the identity and in vitro susceptibility of an infecting microorganism are rarely known when therapy is begun, initial therapy is usually empirical and is based on knowledge of the agents likely to be present and their historical susceptibility (Tables 28.1, 28.2). In some cases, the most likely etiologic agent can be highly suspected simply based on the clinical presentation and the horse's history. For example, abscessation of the submandibular and retropharyngeal lymph nodes is most likely caused by *Streptococcus equi* subspecies *equi*. On the other hand, pleuropneumonia in an adult horse may be caused by any one or combinations of a number of bacteria and thus requires bacteriologic culture of a tracheobronchial aspirate and pleural fluid to determine the etiologic agent(s). A Gram stain of properly collected material is a simple,

rapid and inexpensive means of identifying the presence and morphological features of microorganisms in body fluids that are normally sterile. A negative Gram stain is, however, not sufficient to confirm the absence of microorganisms. Although visualization of bacteria on a Gram stain rarely reveals their identity, it can provide useful information regarding therapy while awaiting bacterial culture and antimicrobial susceptibility testing. For example, Gram-positive cocci in chains suggest *Streptococcus* spp. *Streptococcus* spp. isolated from a purulent lesion in a horse are likely group C streptococci, which are usually susceptible to penicillin. On the other hand, the presence of both Gram-positive and Gram-negative bacteria indicates a mixed infection that will require broad-spectrum antimicrobial agents at least until bacterial culture reveals the etiologic agents and their in vitro susceptibility pattern. The initial selection of the antimicrobial agent and route of administration will depend on the severity of the disease and the site of infection. A combination of gentamicin for Gram-negative coverage and penicillin for Gram-positive and anaerobic coverage is commonly used as initial broad-spectrum therapy for severe bacterial infections in adult horses. Enrofloxacin can be used as a substitute to gentamicin in adult horses, whereas ampicillin or cefazolin can replace penicillin. Addition of metronidazole is recommended for disease processes where *Bacteroides fragilis* is commonly isolated, such as pleuropneumonia and peritonitis.

Susceptibility testing is not necessary for all equine clinical isolates. For example, most β -hemolytic *Streptococci* isolated from horses are susceptible to penicillin G, as are most anaerobes except *Bacteroides fragilis*. *Pasteurella* spp. isolated from horses also have a predictable susceptibility profile (Table 28.1). In con-

trast, Enterobacteraceae, *Pseudomonas* spp., *Enterococcus* spp. and *Staphylococcus* spp. have unpredictable susceptibility (Table 28.1). In vitro susceptibility testing is particularly important for these bacterial species. While the etiologic agent may be susceptible to several antimicrobial agents, not all such agents may reach therapeutic concentrations at the site of infection. The rate and extent of penetration of a drug into sites outside the vascular space are determined by the drug's concentration in plasma, molecular charge and size, lipid solubility and extent of plasma protein binding (Chapter 4). It can also be affected by specific uptake by cells, cellular barriers (e.g., blood-brain barrier) and tissue blood flow. Thus, the goal of antimicrobial therapy is to select an antibiotic that, in addition to exhibiting good antimicrobial activity against the infecting microorganism, will achieve therapeutic concentrations in the infected area.

Determination of the appropriate dose and dosing interval of an antimicrobial agent requires knowledge and integration of its pharmacokinetic and pharmacodynamic properties. The pharmacokinetic properties of a drug describe its disposition within the body and include the processes of drug absorption, distribution, metabolism, and excretion (Chapter 4). On the other hand, pharmacodynamic properties of a drug address the relationship between drug concentration and antimicrobial activity (Chapter 5).

The most significant factor determining the efficacy of beta-lactams, macrolides, tetracyclines, trimethoprim-sulfonamide combinations, chloramphenicol, and glycopeptides is the length of time that serum levels exceed the MIC of the pathogen. Increasing the concentration of the drug several-fold above the MIC does not significantly increase the rate of microbial killing. Rather, it is the length of time that bacteria are exposed to concentrations of these drugs above the MIC that dictates their rate of killing. Optimal dosing of such antimicrobial agents involves frequent administration.

Other antimicrobial agents, such as the aminoglycosides, fluoroquinolones, and metronidazole, exert concentration-dependent killing characteristics. Their rate of killing increases as the drug concentration increases above the MIC for the pathogen, and it is not necessary or even beneficial to maintain drug levels above the MIC between doses. Thus, optimal dosing of aminoglycosides and fluoroquinolones involves administration of high doses at long dosing intervals.

The route of administration and the antimicrobial preparations available also greatly influence the choice of an antimicrobial agent for use in the horse. Intravenous medication is usually restricted to hospitalized horses or those under the direct care of a veterinarian. Maintenance of an intravenous catheter in the field, while certainly possible, is not considered a routine practice. Intramuscular administration of antibiotics to the horse is restricted by duration of treatment and volume of the preparation (total dose) to be administered. Repeated injection of large volumes of medication results in local muscle necrosis and pain. Even well-behaved horses object to repeated injections. Novice horse owners can rarely use the IM site of injection in the rump and thus are limited to rotation of injection sites on both sides of the neck. For this reason, oral administration of antibiotics is the most popular route of administration to horses.

Unfortunately, several antimicrobial agents commonly used orally in other monogastric species, such as penicillin G, amoxicillin, cefadroxil, and ciprofloxacin, are poorly absorbed, particularly in adult horses, and therefore cannot be used orally. The large bowel of the horse makes this species particularly susceptible to antimicrobial-induced enterocolitis secondary to disruption of the normal colonic microflora and overgrowth of pathogenic microorganisms, most likely *Clostridium* spp. including *C. difficile*. The onset of acute and sometimes fatal diarrhea in the horse has been anecdotally associated with the use of almost every oral and parenteral antimicrobial agent. However, orally administered antimicrobials with low bioavailability and good activity against anaerobes are most likely to induce diarrhea. For this reason, oral beta-lactam antimicrobials should be used with caution in the horse. Antimicrobials that are partially excreted in the bile after parenteral administration should also be used with caution. Certain antibiotics such as lincomycin and clindamycin are associated with well recognized enterocolitis syndromes, and their use must be avoided in horses. Other antibiotics such as oral trimethoprim-sulfonamide combinations, erythromycin, chloramphenicol, metronidazole, and parenteral oxytetracycline and cephalosporins have been occasionally linked to enterocolitis in horses. In one study, administration of oxytetracycline to horses was associated with the proliferation of *Clostridium perfringens* type A and possible toxin production (White et al., 1982). Anecdotally, some antibi-

Table 28.1. In vitro antimicrobial susceptibility of selected bacterial isolates from horses.^a

Microorganisms(n ^c)	Antimicrobials ^b																					
	GM	AMI	AZM	CLR	E	CHL	TMS	TE	RIF	P	AM	A/S	OX	TIM	CFZ	CFT	XNL	CPE	IMP	ENR	CIP	VAN
Gram-positive																						
<i>Staphylococcus aureus</i> (243)	64	86	70	86	70	99	73	73	89	32	37	68	72	79	68	68	75 ^d	68	68	94	83	97
Other staphylococci (48)	91	100	76	73	76	97	85	71	97	30	30	65	65	—	65	65	85 ^d	65	65	94	97	100
<i>Rhodococcus equi</i> (114)	100	100	93	96	93	96	75	75	93	—	—	—	—	—	—	—	—	—	91	71	100	100
<i>Streptococcus equi</i> (130)	74	10	99	99	99	100	74	92	96	99	99	—	—	100	100	100	98	—	—	75	—	100
<i>S. zooepidemicus</i> (786)	75	2	97	97	97	100	51	26	98	97	97	—	—	100	100	100	99	—	—	60	—	100
<i>Enterococcus</i> spp. (38)	—	—	—	—	52	83	68	63	60	77	83	—	—	—	—	—	34	—	—	53	80	95
Gram-negative																						
<i>Escherichia coli</i> (505)	75	93	—	—	—	71	47	53	—	—	45	53	—	73	70	87	80	97	100	88	88	—
<i>Klebsiella</i> spp. (168)	66	96	—	—	—	84	57	65	—	—	3	83	—	88	90	98	80	98	100	90	100	—
<i>Enterobacter</i> spp. (183)	54	91	—	—	—	49	51	55	—	—	16	17	—	43	10	53	54	80	100	81	87	—
<i>Salmonella enterica</i> (224)	65	99	—	—	—	64	87	62	—	—	65	65	—	68	—	88	64	98	100	100	100	—
<i>Pseudomonas aeruginosa</i> (48)	70	97	—	—	—	—	—	—	—	—	—	—	—	94	—	15	6	91	97	65	97	—
<i>Pasteurella</i> spp. (40)	94	94	—	—	—	100	91	100	—	—	100	100	—	100	94	100	95	100	100	100	100	—
<i>Actinobacillus</i> spp. (167)	91	76	—	—	—	98	57	86	—	54	74	100	—	100	100	100	98	100	100	95	100	—

^aData from the Clinical Microbiology Laboratory, University of Florida (2003-2005) and the Animal Health Laboratory (AHL), University of Guelph (1999-2004). Data from the AHL was kindly provided by Dr. Beverly McEwen.

^bPercent susceptible isolates.

^cApproximate number of isolates (some isolates were not tested against every antimicrobial agent).

^dIn vivo, ceftiofur is rapidly metabolized to desfuroylceftiofur. Desfuroylceftiofur is as effective as ceftiofur against most bacterial pathogens but most coagulase positive *Staphylococcus* spp. are resistant. Therefore, despite in vitro susceptibility, ceftiofur is not the ideal choice for the treatment staphylococcal infections.

—Not tested or testing not indicated.

GM, gentamicin; AMI, amikacin; AZM, azithromycin; CLR, clarithromycin; E, erythromycin; CHL, chloramphenicol; TMS, trimethoprim-sulfonamide; TE, tetracycline; RIF, rifampin; P, penicillin; AM, ampicillin; A/S, ampicillin/sulbactam; OX, oxacillin; TIM, ticarcillin-clavulanic acid (Timentin); CFZ, cefazolin; CFT, cefotaxime; XNL, ceftiofur; CPE, cefepime; IMP, imipenem; ENR, enrofloxacin; CIP, ciprofloxacin; VAN, vancomycin.

otics are known to induce diarrhea in some parts of the world while used extensively without evidence of such side effect in others. This marked geographic variation in the incidence of antibiotic-induced diarrhea likely results from differences in colonic microflora. Foals seem less susceptible to antibiotic-induced enterocolitis than adult horses.

Bacterial septicemia is the leading cause of morbidity and mortality in neonatal foals. Many of the diseases involving the neonatal foal are the sequelae of septicemia. These diseases include pneumonia, peritonitis, meningitis, osteomyelitis, septic arthritis, and omphalophlebitis. Gram-negative bacteria account for 70% to 95% of the microorganisms isolated from cultures of blood samples in equine neonates, with *Escherichia coli* being by far the most common isolate. Other Enterobacteriaceae (*Klebsiella* spp., *Salmonella* spp., and *Enterobacter* spp.) and nonenteric Gram-negative rods (*Pasteurella* spp. and *Actinobacillus* spp.) are also commonly isolated. Gram-positive cocci (β -hemolytic streptococci, *Enterococcus* spp., and *Staphylococcus* spp.) account for approximately 15% of equine neonatal blood culture isolates at the University of Florida.

Treatment protocols for equine neonates must include antimicrobials with a high level of activity against enteric Gram-negative bacteria while providing adequate coverage against Gram-positive microorganisms. Bactericidal agents are preferred because neonatal foals have a naive immune system and their defense mechanisms against bacterial pathogens are often compromised. The combination of an aminoglycoside (amikacin or gentamicin) with either penicillin or ampicillin is often initiated until culture results are available. Such combination provides adequate coverage against approximately 90% of bacterial isolates recovered from blood cultures at the University of Florida. Amikacin, although more expensive, is preferred to gentamicin because of its lower frequency of resistance amongst Enterobacteriaceae. Similarly, ampicillin is preferred to penicillin because of its higher activity against enterococci. In situations when an aminoglycoside should not be used, such as renal failure, adequate coverage is provided by a third- or fourth-generation cephalosporin such as cefotaxime or cefepime, respectively. If blood or other cultures are positive, antibiotic therapy can then be adjusted according to the susceptibility test results. If the animal has a positive blood culture, a minimum of 2 weeks of

antibiotic therapy is recommended; if the infection is well established in an organ, such as the lung, joints, or bones, then antibiotics should be provided on a long-term basis (Koterba, 1990).

For other non-septicemic bacterial infections, duration of treatment should continue for at least 72 hours after return to clinical normality, and should be considerably longer in the case of life threatening infections such as pleuropneumonia, pulmonary or abdominal abscess, peritonitis, osteomyelitis, or endocarditis. In addition to a thorough physical examination, other diagnostic tests such as hematology, serum biochemistry, radiographs, or ultrasonographic examination may be useful, depending on the disease process, to determine when it is appropriate to discontinue therapy.

The remainder of this chapter outlines major infectious diseases of horses by organ system and provides recommendations for initial selection of antimicrobial agents while awaiting culture and sensitivity results (Table 28.2). Suggested drug dosages are shown in Table 28.3. Once an antimicrobial agent has been selected, the reader should consult the appropriate chapter for potential toxicities and specific contraindications. Very few of the antimicrobial agents mentioned in this chapter have been approved for use in horses. Those that have been approved are often recommended here at higher dosages or to treat a disease other than that for which the compound is approved. For most antibiotics, controlled safety studies involving administration of the drug to a large number of horses have not been performed. It must also be remembered that, although this chapter deals strictly with antimicrobial therapy, supportive, local, or surgical therapy may in some cases be as important as the antibiotic in resolution of the infection. Recommendations for intrauterine therapy are presented in Table 28.4.

Bibliography

- Dowling PM. 2004. Antimicrobial therapy. In: Reed SM, Baily WM, Sellon DC (eds). *Equine Internal Medicine*, 2nd Edition. Philadelphia: Saunders. 186.
- Ensink JM, et al. 1993. In-vitro susceptibility to antimicrobial drugs of bacterial isolates from horses in the Netherlands. *Equine Vet J* 25:309.
- Jacks S, et al. 2003. In vitro susceptibilities of *Rhodococcus equi* and other common equine pathogens to azithromycin, clarithromycin, and 20 other antimicrobials. *Antimicrob Agents Chemother* 47:1742.

Koterba AM. 1990. Antibiotic therapy. In: Koterba AM, et al. (eds). *Equine Clinical Neonatology*. Philadelphia: Lea & Febiger.

Moore RM, et al. 1992. Antimicrobial susceptibility of bacterial isolates from 233 horses with musculoskeletal infection during 1979-1989. *Equine Vet J* 24:450.

White G, Prior SD. 1982. Comparative effects of oral administration of trimethoprim/sulphadiazine or oxytetracycline on the faecal flora of horses. *Vet Rec* 111:316.

Table 28.2. Antimicrobial drug selection in horses.

Site	Diagnosis	Common Infecting Organism(s)	Comments	Suggested Drug(s)	Alternative Drug(s)
Upper respiratory tract	Strangles	<i>Streptococcus equi</i>	Treatment of a horse with strangles depends on the stage of the disease. While the organism is sensitive to penicillin, parenteral antibiotics given after abscess formation may prolong the disease. Horses with severe systemic signs require antibiotics.	Penicillin G ^a	Ceftiofur
	Guttural pouch empyema	<i>Streptococcus equi</i> , <i>S. zooepidemicus</i> , rarely Gram-negatives	Local irrigation with saline is the treatment of choice. Lowering the horse's head facilitates drainage and reduces the risks of aspiration. Systemic or topical antimicrobials rarely indicated unless infection is spreading.	Penicillin G ^a	Ceftiofur; trimethoprim-sulfonamide ^b
	Guttural pouch mycosis	<i>Emmericella nidulans</i> , <i>A. fumigatus</i> , other opportunistic fungi	Surgical therapy is the treatment of choice. Even when successful, medical therapy may be too slow to prevent several bouts of hemorrhage.	Topical enilconazole; systemic antifungal agents usually not required	Topical natamycin
	Fungal rhinitis	<i>Aspergillus</i> spp., other opportunistic fungi	Surgical removal of the mycotic plaque and associated necrotic tissue, combined with topical antifungal therapy.	Topical enilconazole	Topical natamycin; Topical amphotericin B
	Sinusitis, primary	<i>S. zooepidemicus</i> , <i>S. equi</i>	Treatment may consist of daily lavage of sinus with saline (± antiseptics or antimicrobial agents) combined with systemic antimicrobial agents. Nonresponsive cases may require sinusotomy.	Penicillin G ^a	Ceftiofur; trimethoprim-sulfonamide ^b
	Sinusitis, secondary	Mixed opportunistic aerobic ^c and anaerobic ^d infection	Usually requires treatment of primary problem, e.g., extraction of diseased tooth.	Penicillin G ^a	Ceftiofur; trimethoprim-sulfonamide ^b and metronidazole; chloramphenicol
Lung	Bacterial pneumonia or lung abscesses; adults	<i>S. zooepidemicus</i> , opportunistic aerobic pathogens ^c , <i>S. pneumoniae</i> , <i>Mycoplasma</i> spp.	<i>S. zooepidemicus</i> is most commonly isolated.	Broad-spectrum antibiotics ^e Penicillin G ^a is drug of choice if streptococcal infection is confirmed. Oxytetracycline	Ceftiofur; trimethoprim-sulfonamide ^b Enrofloxacin ^f , chloramphenicol
	Bacterial pneumonia or lung abscesses; older foals	<i>S. zooepidemicus</i>	Most common cause of pneumonia/bronchitis in older foals.	Penicillin G ^a	Ceftiofur; trimethoprim-sulfonamide ^b ; erythromycin ± rifampin for <i>S. zooepidemicus</i> abscesses
		Opportunistic aerobic ^c		Broad-spectrum antibiotics ^e	3rd-generation cephalosporins; trimethoprim-sulfonamide ^b

(continued)

Table 28.2. Antimicrobial drug selection in horses. (continued)

Site	Diagnosis	Common Infecting Organism(s)	Comments	Suggested Drug(s)	Alternative Drug(s)
Gastrointestinal	Bacterial pneumonia; neonatal foals	<i>Rhodococcus equi</i>	Treatment must be a minimum of 3-4 weeks.	Rifampin and macrolide (erythromycin, azithromycin or clarithromycin)	Chloramphenicol + rifampin; trimethoprim-sulfonamide + rifampin
		<i>Pneumocystis carinii</i>	May be found in immunocompromised foals or in association with <i>R. equi</i> .	Trimethoprim-sulfonamide	
		Opportunistic aerobic pathogens ^c	Neonatal pneumonia often a part of a generalized infection affecting many different organ systems.	Broad-spectrum antibiotics ^e (amikacin preferred over gentamicin)	3rd-generation cephalosporins; ticarcillin-clavulanic acid
		Opportunistic aerobic ^c and anaerobic pathogens ^d	While systemic antimicrobial agents are most essential treatment for bacterial pleuropneumonia, thoracic drainage and nursing care are important.	Broad-spectrum antibiotics ^e ± metronidazole	Ceftiofur ± metronidazole; enrofloxacin ^f ± metronidazole; trimethoprim-sulfonamide and metronidazole
	Fungal pneumonia	<i>Mycoplasma felis</i>		Oxytetracycline	Enrofloxacin ^f ; chloramphenicol
		Opportunistic fungi: <i>Aspergillus</i> spp., <i>Candida</i> spp., <i>Mucor</i> spp.	If fungal pneumonia is secondary to severe primary disease (i.e., liver failure, enterocolitis, peritonitis), treatment is difficult and prognosis is poor. If fungal pneumonia is secondary to aggressive antibiotic therapy (i.e., neonatal foal) then prognosis is guarded.	Amphotericin B	Itraconazole
	Tuberculosis	<i>Mycobacterium</i>	Treatment is not usually attempted. Public health concern. Reportable disease.	See Chapter 23	
	Oral, gastric candidiasis	<i>Candida</i> spp.	Seen in immunosuppressed animals or ones on long-term antibiotic therapy. May just require discontinuation of antibiotic therapy.	Fluconazole	Amphotericin B; itraconazole
	Acute enterocolitis; salmonellosis	<i>S. typhimurium</i> , other serovars	Systemic antimicrobials indicated in animals showing signs of or at risk for septicemia (foals, immunocompromised animals, aged animals). Treatment with antibiotic is not thought to alter the course of the disease.	Broad-spectrum antibiotics ^e ; enrofloxacin ^f	3rd-generation cephalosporins; susceptibility variable
	Acute enterocolitis; clostridiosis	<i>C. difficile</i> , <i>C. perfringens</i> type A, <i>C. perfringens</i> type C	The first approach in therapy is to stop the precipitating antimicrobial agent when applicable.	Metronidazole	Oral bacitracin (22 mg/kg PO BID day 1, then SID); oral vancomycin ^g
	Potomac horse fever (equine ehrlichial colitis)	<i>Neorickettsia risticii</i>		Oxytetracycline	Oral doxycycline ^h ; rifampin and erythromycin
	Proliferative enteropathy	<i>Lawsonia intracellularis</i>	Proliferative ileitis and diarrhea in foals.	Erythromycin ± rifampin	Oxytetracycline; doxycycline ^h ; chloramphenicol
Abdominal abscess		<i>S. equi</i> , <i>S. zooepidemicus</i> , <i>Corynebacterium pseudotuberculosis</i>	Most commonly a complication of strangles. Long-term treatment frequently required.	Penicillin G ^a ± rifampin	Erythromycin ± rifampin; chloramphenicol; trimethoprim-sulfonamide ^o
		<i>R. equi</i> (foals)	Abdominal abscess(es) and ulcerative enterocolitis. Peritonitis may be present. Pneumonia, diarrhea, septic phytitis or arthritis may occur concurrently.	Rifampin and macrolide (erythromycin, clarithromycin, or azithromycin)	Chloramphenicol ± rifampin; Trimethoprim-sulfonamide + rifampin

Table 28.2. Antimicrobial drug selection in horses. (continued)

Site	Diagnosis	Common Infecting Organism(s)	Comments	Suggested Drug(s)	Alternative Drug(s)
	Peritonitis	Mixed opportunistic aerobic ^c and anaerobic pathogens ^d , <i>Actinobacillus equuli</i>	Obtaining culture and sensitivity of peritoneal fluid highly recommended. Peritoneal lavage may be beneficial in some cases.	Broad-spectrum antibiotics ^a and metronidazole	3rd- or 4th-generation cephalosporin and metronidazole; penicillin G ^a + enrofloxacin ^f + metronidazole
	Tyzer's disease	<i>Clostridium piliforme</i>	Treatment is usually not successful.	Erythromycin ± rifampin; penicillin G and aminoglycoside	Oxytetracycline
	Liver abscess	β-hemolytic Streptococci, <i>C. pseudotuberculosis</i> , Opportunistic aerobic ^c or anaerobic ^d pathogens	Ultrasound may be helpful in diagnosis. May occur concurrently with other abdominal abscess(es). Long-term treatment required.	Broad-spectrum antibiotics ^a combination with rifampin or metronidazole	
	Cholangiohepatitis	Gram-negative enteric organisms	May be difficult to identify the offending organism(s). Long term therapy required. Prognosis is poor when several obstructing calculi are present. For obstructing stones, choledocholithotomy may be indicated.	Enrofloxacin ^f	Ceftiofur; trimethoprim-sulfonamide
Soft tissue	Candidiasis	<i>Candida</i> spp.	Infection of multiple systems may occur. Fungemia, although uncommon, has been seen in immuno-compromised foals on aggressive, broad-spectrum antibiotic therapy.	Fluconazole	Amphotericin B; itraconazole
	Bacterial septicemia	<i>E. coli</i> , opportunistic aerobic ^c pathogens (mostly Gram-negatives)	Neonate is most commonly affected. Parenteral administration of antibiotics recommended, at least initially. Treatment required for a minimum of 2 weeks.	Broad-spectrum antibiotics ^a (amikacin preferred over gentamicin)	3rd- or 4th-generation cephalosporins; ticarcillin-clavulanic acid
	Omphalophlebitis	Opportunistic aerobic ^c pathogens	Diagnostic ultrasound is useful when external signs of infection are not apparent. Surgical resection may be the treatment of choice in some cases.	Broad-spectrum antibiotics ^a (amikacin preferred over gentamicin)	3rd- or 4th-generation cephalosporins; ticarcillin-clavulanic acid
	Fistulous withers	<i>Brucella abortus</i> , <i>Actinomyces bovis</i>	Public health concern with brucellosis. Treatment regimen using killed <i>Brucella</i> vaccine may be effective.	Oxytetracycline and streptomycin or gentamicin	Oral doxycycline ^b or trimethoprim-sulfonamide and gentamicin or rifampin
		Opportunistic aerobic ^c and anaerobic ^d pathogens		Broad-spectrum antibiotics ^a	Ceftiofur; trimethoprim-sulfonamide
	Traumatic and contaminated wounds	Opportunistic aerobic ^c and anaerobic ^d pathogens	Exploration, lavage, debridement and local therapy are more important than systemic antimicrobial agents.	Trimethoprim-sulfonamide (superficial wound); Broad-spectrum antibiotics ^a (deep contaminated wound)	Ceftiofur
	Ulcerative lymphangitis	<i>C. pseudotuberculosis</i>	Drainage of <i>C. pseudotuberculosis</i> subcutaneous abscesses is preferred over antibiotic therapy. Systemic antibiotics required for ulcerative lymphangitis, internal abscesses, or in horses with signs of systemic illness.	Penicillin G ^a	Trimethoprim-sulfonamide; Erythromycin ± rifampin; chloramphenicol
	Subcutaneous abscesses	β-hemolytic <i>Streptococcus</i> spp.	Drainage of abscesses preferred over antibiotic therapy. Systemic antibiotics required for internal abscesses or in horses with signs of systemic illness.	Penicillin G ^a	Ceftiofur; chloramphenicol

(continued)

Table 28.2. Antimicrobial drug selection in horses. (continued)

Site	Diagnosis	Common Infecting Organism(s)	Comments	Suggested Drug(s)	Alternative Drug(s)
	Burns	<i>P. aeruginosa</i> , <i>S. aureus</i> , Other opportunistic Aerobic ^c pathogens	Care of burn wounds includes thorough cleansing, surgical debridement, daily hydrotherapy, and topical antimicrobials. Systemic antibiotics are not effective in preventing local burn wound infections and may permit the growth of resistant bacteria. Systemic antibiotics only if signs of systemic infection.	Topical: silver sulfadiazine cream; Systemic: broad-spectrum antibiotics ^e	Ticarcillin-clavulanic acid; 3rd-generation cephalosporins
Bone and joint	<i>Clostridium</i> myonecrosis	<i>C. perfringens</i> , <i>C. septicum</i> , <i>C. chauvoei</i> , other spp.	Surgical debridement, including fasciotomy, and supportive care are essential. Poor prognosis.	Penicillin G (IV every 4 hours)	Metronidazole; chloramphenicol
	Osteomyelitis; septic arthritis neonates	Opportunistic aerobic ^c pathogens, <i>Salmonella</i> spp., <i>R. equi</i>	In foals, osteomyelitis and septic arthritis are seen secondary to septicemia. Antibiotics and surgical debridement are required for osteomyelitis. Antibiotics and joint lavage are required for septic arthritis. Intra-articular antibiotics as well as regional or intraosseous perfusion with antimicrobial may be beneficial.	Broad-spectrum antibiotics ^e (amikacin preferred over gentamicin); See above for <i>R. equi</i>	3rd- or 4th-generation cephalosporins; ticarcillin-clavulanic acid
	Osteomyelitis adults	Opportunistic aerobic ^c pathogens	Usually secondary to traumatic and contaminated wounds. Antibiotics and surgical debridement are required.	Broad-spectrum antibiotics ^e	3rd- or 4th-generation cephalosporins; trimethoprim-sulfa
	Septic arthritis or tenosynovitis adults	<i>Staphylococcus</i> spp., opportunistic aerobic ^c pathogens	In adults, septic arthritis is usually associated with intra-articular injection or wounds. Joint/tendon sheath drainage and lavage are highly recommended. Intra-articular antibiotics as well as regional or intraosseous perfusion with antimicrobials may be beneficial. In vitro susceptibility testing highly recommended.	1 st generation cephalosporin and amikacin or gentamicin	Broad-spectrum antibiotics ^e ; trimethoprim-sulfonamide
Skin	Lyme disease	<i>Borrelia burgdorferi</i>	Definitive diagnosis is difficult; Presence of serum antibody does not indicate disease.	Oxytetracycline	Oral doxycycline ^h ; ceftriaxone; ceftiofur
	Dermatophilosis (streptothricosis, rain rot)	<i>D. congolensis</i>	Systemic therapy often unnecessary and generally reserved for severe or generalized cases. Infected animals should be groomed and bathed daily with povidone-iodine shampoo or chlorhexidine solution (Novalsan 2%). If treated systemically a short course of antibiotics is often effective (3-5 days).	Procaine penicillin G	Ampicillin
	Folliculitis/furunculosis	<i>Staphylococcus</i> spp., <i>Streptococcus</i> spp., <i>C. pseudotuberculosis</i>	Same as dermatophilosis. Antibiotics, if required, should be based on culture/sensitivity.	Broad-spectrum antibiotics ^e	Trimethoprim-sulfonamide
	Staphylococcal cellulitis	<i>S. aureus</i> , <i>S. intermedius</i>	Requires aggressive systemic antibiotics.	1 st generation cephalosporin and gentamicin or amikacin (amikacin preferred)	Broad-spectrum antibiotics ^e ; trimethoprim-sulfonamide; chloramphenicol
	Dermatophytosis	<i>Trichophyton equinum</i> , <i>T. mentagrophytes</i> , <i>Microsporum gypsum</i> , <i>M. equinum</i> , etc.	Disease may spontaneously regress but therapy shortens the recovery period and may decrease the spread of the disease. Topical therapy is sufficient. Treat the whole body of all contact animals.	5% lime sulfur or 0.5% sodium hypochloride solution or povidone-iodine topically daily for 3-5 days and reapply weekly until resolution of infection.	Topical natamycin; topical enikonazole; Topical miconazole

Table 28.2. Antimicrobial drug selection in horses. (continued)

Site	Diagnosis	Common Infecting Organism(s)	Comments	Suggested Drug(s)	Alternative Drug(s)
	Sporotrichosis	<i>Sporothrix schenckii</i>	Treatment is often effective. Continue treatment for several weeks after lesions disappear or relapse will occur. Systemic iodides may cause abortion in pregnant mares.	Itraconazole and sodium iodide: 40 mg/kg of 20% solution IV for 2-5 days followed by oral potassium iodide: 2 mg/kg SID PO until lesions regress.	Fluconazole
	Pythiosis (phycomycosis, swamp cancer, Florida horse leech, bursattii, gulf coast fungus)	<i>Pythium insidiosum</i>	Immediate radical surgical removal of all infected tissues is essential for effective treatment. Early immunotherapy with soluble <i>Pythium</i> antigens ⁱ is effective, especially when combined to surgical removal.	Topical or intralesional micronazole; systemic iodides (see sporotrichosis)	Fluconazole
Renal	Cystitis	Opportunistic aerobic ^c bacteria, <i>Candida</i> spp.	Cystitis is usually secondary to urolithiasis, bladder neoplasia or bladder paralysis. Treat for 7-10 days and reculture urine.	Trimethoprim-sulfonamide; fluconazole for <i>Candida</i> spp.	Ceftiofur; broad-spectrum antibiotics ^e
	Pyelonephritis	Opportunistic aerobic ^c bacteria	Same predisposing factors as cystitis. Usually chronic and insidious, may be difficult to treat. Use aminoglycosides cautiously in face of renal disease. Treat a minimum of 2-3 weeks; duration required is variable and may be longer.	Trimethoprim-sulfonamide; 3rd-generation cephalosporins	penicillin G and enrofloxacin ^f
Cardiovascular	Bacterial endocarditis	<i>Streptococcus</i> spp., opportunistic aerobic ^c pathogens	Prognosis is poor to grave. Long term treatment is required (several months). Antibiotic choice should be based on blood culture.	Broad-spectrum antibiotics ^e ± rifampin	3rd- or 4th-generation cephalosporin; penicillin G and enrofloxacin ^f
	Bacterial pericarditis	<i>Streptococcus</i> sp., mixed opportunistic aerobic ^c and anaerobic ^d pathogens	Prognosis is guarded. Culture of pericardial fluid is recommended. Drainage and lavage of the pericardial sac are also recommended.	Broad-spectrum antibiotics ^e	3rd- or 4th-generation cephalosporin; penicillin G and enrofloxacin ^f
	Thrombophlebitis	Mixed opportunistic aerobic and anaerobic pathogens	Blood culture recommended.	Broad-spectrum antibiotics ^e ± metronidazole	Ceftiofur; trimethoprim-sulfonamide
Nervous	Bacterial meningitis or spinal abscess	Opportunistic aerobic ^c pathogens	Most often associated with neonatal septicemia. Prognosis is poor.	3rd- or 4th-generation cephalosporin ± aminoglycoside (amikacin preferred)	Broad-spectrum antibiotics ^e ; penicillin G and enrofloxacin ^f ; trimethoprim-sulfonamide
	Mycotic meningitis/encephalitis	<i>Cryptococcus neoformans</i> <i>Aspergillus</i> spp.	Prognosis is grave.	Fluconazole	Amphotericin B
	Brain abscess	<i>Streptococcus equi</i> , <i>Streptococcus</i> spp.	Prognosis is grave.	Amphotericin B Penicillin G ^a ± rifampin	Itraconazole 3rd-generation cephalosporin;
	Tetanus	<i>Clostridium tetani</i>	Antibiotics to eliminate the infection but tetanus antitoxin to neutralize the unbound toxin.	Metronidazole	Ampicillin; Penicillin G
	Botulism	<i>Clostridium botulinum</i>	Antitoxin to neutralize unbound toxin. Antibiotics if suspected wound contamination or to prevent complications such as aspiration pneumonia.	Penicillin G ^a	Ampicillin
	Otitis media/interna	<i>Actinobacillus</i> spp. <i>Staphylococcus</i> spp., <i>Streptococcus</i> spp., Opportunistic aerobic ^c pathogens	Cause vestibulocochlear and/or facial nerve dysfunction as well as head shaking.	Trimethoprim-sulfonamide	Chloramphenicol; 3rd-generation cephalosporin

(continued)

Table 28.2. Antimicrobial drug selection in horses. (continued)

Site	Diagnosis	Common Infecting Organism(s)	Comments	Suggested Drug(s)	Alternative Drug(s)
Ophthalmic	Equine protozoal myeloencephalitis	<i>Sarcocystis neurona</i>	Treatment may stop progression of disease and occasionally reverse clinical signs. Long term therapy required.	Ponazuril	Sulfadiazine (24 mg/kg PO SID) and pyrimethamine (1 mg/kg PO SID); nitazoxamide
	Bacterial keratitis; Mild corneal ulceration	Gram-negative or Gram-positive opportunistic bacteria	Topical application.	Topical bacitracin-neomycin-polymyxin B combinations	Topical gentamicin
	Bacterial keratitis; Severe melting keratitis	<i>P. aeruginosa</i>	Topical (or subconjunctival when appropriate) application (see Chap. 22).	Topical tobramycin; topical amikacin	Topical ciprofloxacin
	Fungal keratitis	<i>Aspergillus</i> spp., <i>Alternaria</i> spp., <i>Mucor</i> spp., <i>Fusarium</i> sp., <i>Candida</i> spp.	Topical application.	Natamycin	Miconazole; itraconazole-DMSO ointment; silver sulfadiazine
	Foreign body penetration	Gram-negative or Gram-positive bacteria, fungal agents	Topical broad-spectrum coverage. Systemic antimicrobials indicated if anterior chamber penetrated and/or if peri-orbital tissues are infected.	Topical gentamicin; systemic broad-spectrum antibiotics ^a	Topical tobramycin; systemic trimethoprim-sulfonamide; penicillin G and enrofloxacin ^f
	Manifestation of systemic disease	Bacterial: <i>A. equuli</i> , <i>Leptospirosis</i> , <i>R. equi</i>	Ocular signs may be immune-mediated. Primary treatment is aimed at systemic disease.	See specific infection	
		Fungal: <i>Cryptococcus</i> spp., <i>Histoplasma</i> spp., <i>Aspergillus</i> spp.	Often associated with optic neuritis, chorioretinitis, anterior uveitis, blepharitis, purulent conjunctivitis.	See specific infection	
Reproductive tract	Retained placenta	<i>S. zooepidemicus</i> , coliforms	Bacterial infections are commonly associated with prolonged (>6-8 hours) retention of membranes. Systemic antimicrobials recommended if early treatment with oxytocin fails.	Broad-spectrum antibiotics ^a ± metronidazole	Trimethoprim-sulfonamide; 3rd-generation cephalosporin
	Endometritis, metritis, and pyometra	<i>S. zooepidemicus</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i>	Control of pneumovagina (Caslick's) is indicated in most cases. Urovagina and peritoneal lacerations also predispose to infection. Antiseptics used by the intrauterine route may induce a chemical irritation. Uterine lavage and hormonal therapy (e.g., oxytocin, PGF ₂) are adjunct treatments. Systemic antibiotics are indicated primarily when endometrial biopsy suggests a deep endometrial infection or in cases of septic metritis with systemic clinical signs. Therapy based on <i>in vitro</i> susceptibility testing.	Intrauterine therapy daily for 3-5 days during estrus or early diestrus (Table 28.4) Choice of agent based on culture and sensitivity.	
	Fungal endometritis	<i>Candida</i> spp., <i>Aspergillus</i> spp.	Systemic antifungal agents are usually not warranted. Intrauterine therapy based on <i>in vitro</i> susceptibility testing. Daily lavage with 1% iodine or 1% acetic acid may be sufficient.	Intrauterine: clotrimazole	Intrauterine: nystatin; amphotericin B
	Placentitis	Highly variable. <i>S. zooepidemicus</i> , <i>E. coli</i> , <i>Klebsiella</i> spp., are most common	Culture and sensitivity of discharge is highly recommended as organism(s) involved are unpredictable. It may be difficult to obtain effective antibiotic levels at the site of infection and resolution of the infection may not be possible until after parturition.	Broad-spectrum antibiotics ^a	Trimethoprim-sulfonamide

Table 28.2. Antimicrobial drug selection in horses. (continued)

Site	Diagnosis	Common Infecting Organism(s)	Comments	Suggested Drug(s)	Alternative Drug(s)
	Contagious equine metritis	<i>Taylorella equigenitalis</i>	Mares may become carriers once infected. Stallions are asymptomatic carriers. Reportable disease.	Mares: intrauterine potassium penicillin, cleansing of vulva and clitoral fossa with 4 % chlorhexidine solution followed with packing of the clitoral fossa with chlorhexidine or nitrofurazone ointment. Stallions: potassium penicillin G, 2000 IU per ml of semen extender. Wash penis daily with chlorhexidine solution and pack with nitrofurazone ointment.	
	Mastitis	<i>S. zooepidemicus</i> , <i>Staphylococcus</i> spp., Other opportunistic aerobic ^c pathogens, <i>Mycoplasma</i> spp.	Systemic antimicrobial therapy is recommended. Intramammary preparations for cows may also be used.	Trimethoprim-sulfonamide; oxytetracycline for <i>Mycoplasma</i> sp.	Broad-spectrum antibiotics ^e
	Balanoposthitis	<i>S. zooepidemicus</i> , <i>Pseudomonas</i> spp., <i>Klebsiella</i> spp.	Bacterial balanoposthitis as a clinical problem is uncommon. Antimicrobial therapy is directed at infected semen or the recipient mare through the use of antimicrobials in semen extender. Recipient is infused with treated extender immediately prior to natural service. Washing of penis and prepuce with a mild soap is recommended. Disinfectant or topical antibiotics should not be used routinely as recolonization may occur and this treatment may displace commensals and allow pathogens to become established.	Potassium penicillin G 1000 IU and amikacin 1000g per ml of semen extender	Ticarcillin 1000 g per ml of semen extender
	Seminal vesiculitis	<i>P. aeruginosa</i> <i>K. pneumoniae</i> <i>Streptococcus</i> spp. <i>Staphylococcus</i> spp.	Systemic antibiotics based on in vitro susceptibility testing. Antibiotics can also be deposited in the seminal vesicle after lavage using a flexible endoscope. If infection cannot be eradicated, appropriate semen extender must be used for breeding (see recommendations for balanoposthitis).	Penicillin G and enrofloxacin ^f ; trimethoprim-sulfonamide	Ticarcillin-clavulanic acid; broad-spectrum antibiotics ^e
	Orchitis, epididymitis	<i>S. zooepidemicus</i> , <i>K. pneumoniae</i>	In vitro susceptibility testing is recommended.	Broad-spectrum antibiotics ^e	3rd-generation cephalosporins; trimethoprim-sulfonamide
Systemic diseases	Leptospirosis	<i>L. interrogans</i> , serovar Bratislava, Pomona, and others	Uveitis, nephritis, abortions, pyrexia, liver dysfunction.	Oxytetracycline	Ampicillin; doxycycline, penicillin
	Equine ehrlichiosis	<i>Anaplasma phagocytophilum</i>	Fever, limb edema, petechiation, ataxia, anemia, leukopenia, thrombocytopenia.	Oxytetracycline	Oral doxycycline ^h
	Systemic mycosis	<i>Histoplasma capsulatum</i> , <i>Blastomyces dermatidis</i> <i>Coccidioides immitis</i>		Itraconazole Fluconazole	Amphotericin B; Fluconazole Itraconazole

^aPenicillin G (potassium, sodium, or procaine).

^bSome streptococci may be resistant to trimethoprim-sulfonamide in vivo despite in vitro susceptibility.

^cIncludes *E. coli*, *Pasteurella* sp., *Actinobacillus* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*, *Enterobacter* spp., *Proteus* spp., *Staphylococcus aureus*, *S. zooepidemicus*.

^dIncludes *Bacteroides* spp., *Fusobacterium* spp., *Clostridium* spp., *Peptostreptococcus* spp., and others.

^eCombination of a beta-lactam (penicillin G, ampicillin, or a first generation cephalosporin) and an aminoglycoside (gentamicin or amikacin).

^fShould not be used in young growing horses because of the risk of arthropathy.

^gThe use of vancomycin should be restricted to severely ill cases with confirmed *Clostridium* spp. Infection resistant to conventional antimicrobials.

^hAdminister orally only. Intravenous doxycycline has resulted in severe cardiovascular effects including collapse and death in some horses.

ⁱPan American Veterinary Labs (www.pavlab.com), Hutto, Texas.

^jAs opposed to most other 3rd-generation cephalosporins, ceftiofur does not cross the normal blood brain barrier.

Table 28.3. Common antimicrobial drug dosages in horses.^a

Drug preparation	Dose (mg/kg)	Dose interval (h)	Route of administration
β-Lactams			
Benzyl penicillins			
Penicillin G (Na or K)	25,000 IU/kg	6	IV
Penicillin G (procaine)	25,000 IU/kg	12	IM
Aminobenzyl penicillins			
Ampicillin sodium	20	6-8	IV or IM
Ampicillin trihydrate	20	12-24	IM
	20	8	PO (foals only)
Amoxicillin trihydrate	30	8	PO (foals only)
Bacampicillin	25	12	PO
Pivampicillin	25	12	PO
Antistaphylococcal penicillins			
Oxacillin	25	8-12	IM
	25	6	IV
Antipseudomonal penicillins			
Ticarcillin	50	6	IV
Ticarcillin-clavulanic acid	50	6	IV
1st-generation cephalosporins			
Cefazolin	10-20	8	IM
	10-20	6	IV
Cephalothin	20	8	IM
	20-30	6	IV
Cephapirin	20	8	IM
	20	6	IV
Cephalexin	10	6	IV
	30	8	PO
Cephadrine	25	6	IV
	25	6-8	PO (foals only)
Cefadroxil	20-40	8	PO (foals only)
2nd-generation cephalosporins			
Cefoxitin	20	6	IV or IM
3rd-generation cephalosporins			
Cefoperazone	30	6-8	IV or IM
Cefotaxime	40	6	IV
Ceftiofur	2.2-5	12-24	IM
Ceftriaxone	25	12	IV or IM
Cefpodoxime	10	8	PO
4th-generation cephalosporins			
Cefepime	11	8	IV (foals)
	2.2	8	IV (adults)
Carbapenems			
Imipenem ^g	15	6	IV ^c
Aminoglycosides			
Amikacin	10	24	IV or IM (adults)
	25	24	IV or IM (foals)
Gentamicin	6.6	24	IV or IM (adults)
	12	24	IV or IM (foals)

Table 28.3. Common antimicrobial drug dosages in horses.^a (continued)

Drug preparation	Dose (mg/kg)	Dose interval (h)	Route of administration
Fluoroquinolones			
Ciprofloxacin ^b	5.5	24	IV
Enrofloxacin ^b	5.5	24	IV
	7.5	24	PO
Orbifloxacin ^b	7.5	24	PO
Marbofloxacin ^b	2	24	IV or PO
Difloxacin ^b	7.5	24	PO
Moxifloxacin ^b	5.8	24	PO
Fleroxacin ^b	5	24	IV or PO
Tetracyclines			
Oxytetracycline	6.6	12	IV ^c
Doxycycline	10	12	PO ^d
Macrolides			
Erythromycin (phosphate, stearate, ethylsuccinate, estolate)	25	6-8	PO
Erythromycin (lactobionate, gluceptate)	5	6	IV ^c
Azithromycin	10	24-48 ^e	PO
Clarithromycin	7.5	12	PO
Other			
Chloramphenicol (palmitate or base)	50	6 or 12 ^f	PO
Chloramphenicol (sodium succinate)	25-50	6 or 12 ^f	IV
Metronidazole	25	12	PO
	35	12	Per rectum
Tinidazole	15	12	PO
Rifampin	5	12	PO
Sulfadiazine	24	12-24	PO
Trimethoprim-sulfonamide	30 (combined)	12	PO or IV
Pyrimethamine	1	24	PO
Vancomycin ^g	4.5-7.5	8	IV ^h
Sodium iodide (20 % solution)	20-40 ⁱ	24	IV ⁱ
Potassium iodide	10-40 ⁱ	24	PO ⁱ
Antifungal agents			
Amphotericin B	0.5-0.9 ^j	24	IV ^k
Fluconazole	14	loading dose	PO
	5	24	PO
Itraconazole	5	12-24	PO
Ketoconazole	30 (in 0.2N HCl)	12	Intra-gastric ^l
Voriconazole	4	24	PO

^aPharmacokinetics data are available for horses but in most cases safety studies have not been performed in the equine species.

^bShould not be used in young growing horses because of the risk of arthropathy.

^cDilute and give by slow IV infusion.

^dAdminister orally only. Intravenous doxycycline has resulted in severe cardiovascular effects, including collapse and death in some horses.

^eOnce a day for 5 days followed by q 48h therapy.

^fAdminister BID in foals less than 5 days of age and QID thereafter.

^gShould be used only for the treatment of serious bacterial infections caused by microorganisms resistant to all other antimicrobial agents.

^hDilute and administer over 1 hour.

ⁱMay cause abortion in pregnant mares.

^jPharmacokinetic studies are not available. Empirical dose based on human dose, measurement of serum levels in clinical cases, or anecdotal observation of positive clinical response in equine patients.

^kDilute in 5% dextrose and give over 2-4 hours.

^lAdminister by nasogastric tube to prevent irritation by 0.2N HCl.

Table 28.4. Suggested doses for intrauterine antimicrobial therapy in mares.

Drug	Spectrum	Dose ^a
Amikacin sulfate	Excellent Gram-negative coverage (including most <i>P. aeruginosa</i>)	2 g ^b
Gentamicin sulfate	Gram-negative	1-2 g ^b
Enrofloxacin	Gram-negative	1 g
Ticarcillin	Broad-spectrum (not active against <i>Klebsiella</i>)	6 g
Ticarcillin-clavulanic acid	Broad-spectrum	6 g
Penicillin G (potassium)	Gram-positive	5 x 10 ⁶ IU
Ampicillin	Gram-positive	1-3 g ^c
Ceftiofur	Broad-spectrum (not active against <i>P. aeruginosa</i>)	1 g
Nystatin	<i>Candida</i> spp.	500,000 IU
Miconazole	Broad antifungal	200 mg
Clotrimazole	Broad antifungal	500 mg
Amphotericin B	Broad antifungal	200 mg ^d

^aAdminister daily for 3-5 days. The volume infused is determined by the size of the uterus (35-150 ml is usually sufficient).

^bBuffered with equal volume of 7.5% bicarbonate and diluted in saline.

^cUse at high dilutions because it can be irritating.

^dDilute in 100 to 200 ml of sterile water. Makes a relatively insoluble suspension.

Antimicrobial Drug Use in Dogs and Cats

A. David J. Watson, Jane E. Sykes

Veterinarians who treat dogs and cats now work in a wide variety of situations, from rural one-person practices with limited small animal caseloads to specialized multi-veterinarian canine and feline hospitals and advanced referral centers. The range of facilities and services available, and the clients' ability to pay, is correspondingly varied, although the veterinarian's goal remains the same—to provide effective, safe and economical attention for patients in their care. Antimicrobial drugs will often form part of the treatment regimen chosen, but the decision to use them must not be made lightly: these are not placebos or antipyretic agents, nor do they replace the fundamental diagnostic skills of history taking, physical examination, and logical analysis of findings.

Concerns continue in the community about the use and possible misuse of antimicrobial drugs in animals and humans and the potential for adverse outcomes. There is little evidence at present that therapy in dogs and cats poses important threats to human health, but a policy of selective and restricted use is advisable nevertheless to avoid potential criticisms and externally imposed restrictions on their use. There is in any case little doubt that widespread, inappropriate use of these agents can reduce efficacy and create problems with infections that are more difficult and expensive to treat. This has been demonstrated repeatedly by outbreaks of nosocomial infections in human and veterinary hospitals, attributable in part to selection pressure applied by overuse of antimicrobials, especially for prophylaxis in surgical and nonsurgical patients.

Antimicrobial Prophylaxis

The principles and practice of antimicrobial prophylaxis are described in Chapter 21.

Antimicrobial Prophylaxis in Surgery

There are few situations where prophylaxis is warranted in small animal surgery, but Table 29.1 lists some potential indications. The drugs selected should be effective against coagulase-positive *Staphylococcus* spp. and *Escherichia coli*, the microbes most likely to cause postoperative wound infections in dogs and cats. Cefazolin usually has excellent activity against both and has low toxicity. It is also active against many obligate anaerobes, but less so than cefoxitin or cefotetan (both cephamycins are more expensive), which might be preferred if anaerobes are of particular concern, as in colonic and rectal surgery. Where available, injectable amoxicillin-clavulanate and ampicillin-sulbactam preparations are economical alternatives to cephalosporins.

Antimicrobial Prophylaxis in Nonsurgical Patients

Prophylactic use of antimicrobial drugs in nonsurgical patients is controversial and veterinary data are limited. It is not generally warranted, but might benefit individuals considered at high risk because of episodic exposure to an acute transmissible infection, or if local or systemic defenses are compromised severely by disease, investigations, or treatment. Chemoprophylaxis might be effective if the period of risk is brief (a few hours or days), as with some chemotherapy-induced myelosuppression, or the target is a single drug-susceptible species that is unlikely to become resistant, but attempts at long-term chemoprophylaxis are liable to failure. A better approach is to carefully monitor individuals at risk for signs of infection and to treat promptly and appropriately if infection occurs.

Table 29.1. Surgical procedures that may warrant antimicrobial prophylaxis.

Alimentary tract	Dental procedures combined with other surgery; biliary surgery if infection present; resection of esophagus, stomach in gastric dilation-volvulus, or intestine in obstruction; colonic, rectal and anal surgery.
Orthopedic	Extensive internal fracture fixation, open fracture repair, total hip prosthesis.
Other procedures	Perineal herniorrhaphy, hernia repairs with nonabsorbable mesh, pacemaker implantation, lobectomy in infection, extensive neurosurgery, prolonged (>2 hours) surgery with much tissue manipulation.

Antimicrobial Chemotherapy

The principles governing selection and therapeutic use of antimicrobial drugs described in Chapter 6 apply also in treating dogs and cats. Of prime importance in deciding whether and how to treat is an adequate clinical assessment, which should identify the bodily systems involved and the pathogens likely to be responsible. There is a tendency for inexperienced clinicians to assume an increased rectal temperature indicates an infection, but this is not necessarily so. Increased temperatures also occur in other pathologic conditions (neoplasms, drug reactions, immune-mediated disorders, other nonspecific inflammations) and some physiologic states (exercise, excitement, high ambient temperature and humidity). Likewise, leukocytosis is not pathognomic for infection, and can occur also with nonspecific inflammatory processes, hemolysis, neoplasms, trauma, excitement, stress, and glucocorticoid administration.

If a bacterial infection is suspected, an important early decision is whether to undertake in vitro culture and susceptibility testing (see Chapter 2). This usually provides the best guide to the pathogens present and their susceptibility to drugs. It is advisable especially in very serious, recurrent or nonresponsive infections, or whenever the susceptibility profile of the likely pathogens is unknown or unpredictable. In these situations, minimum inhibitory concentrations (MICs) should ideally be sought to help select the most appropriate antimicrobial drug. For lower urinary tract infections, however, serum MICs are not helpful and urinary

MICs must be requested to account for the effect of drug concentration within urine.

Samples for laboratory submission should be taken before any antimicrobial drugs are given. If the infection is serious or life-threatening, treatment should commence immediately, with the option of changing the medication when results become available. Alternatively, one could withhold therapy for 1-2 days pending results, provided the delay is unlikely to be harmful.

Culture and susceptibility testing is not always essential: for example, obligate anaerobic bacterial pathogens and *Streptococcus* spp. have a relatively predictable pattern of susceptibility, so testing is not generally required for them. Furthermore, laboratory testing might be unnecessary for routine or less serious infections in patients that are otherwise healthy. Culture and susceptibility testing may not be feasible in some situations because of lack of funds or facilities.

In these latter two circumstances, information on the likely pathogen can be sought by other means. Firstly, if the infection site is accessible, examination of smears of fine needle aspirates treated with Gram stain and a Romanovsky method (Giemsa, Diff-Quik) can indicate whether bacteria are present and whether they are rods or cocci, and Gram-positive or Gram-negative. A decision on possible treatments can then be made by considering these findings alongside the known spectrum of activity of various antimicrobial drugs.

Alternatively, in the absence of other information, drug selection can be based on knowledge of the site of infection, the pathogens more frequently implicated there, and the likely drug susceptibility of those organisms. This process can be based on personal experience or on published information like that shown in Table 29.2 for dogs and Table 29.3 for cats. In many routine or less serious infections, treatment on this "best-guess" basis will prove satisfactory, without the need for additional investigation.

Whichever selection method is used, it may be necessary to choose between several drugs likely to be effective against the pathogen. All other things being equal, the drug with the narrowest antimicrobial spectrum should be preferred, so as to reduce effects on untargeted microflora. Cost, route of administration, and toxic potential are other factors to consider. Examples of toxic potential that might lead to selection of alternate drugs include, although rare, the

Table 29.2. Antimicrobial drug selection in canine infections.^a

Site or Type	Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs	Alternatives
Skin and subcutis	Deep pyoderma, superficial pyoderma	<i>Staphylococcus</i> , secondary <i>Escherichia</i> , <i>Proteus</i> , <i>Pseudomonas</i>	Seek, remove underlying causes. Anti-staphylococcal drugs often succeed even if other bacteria also present. Prolonged or intermittent dosage may be needed.	Isoxazolyl penicillin ^b , lincosamide ^c , macrolide ^d , oral cephalosporin ^e	Amoxicillin-clavulanate, chloramphenicol, sulfonamide-trimethoprim ^f
	Surface pyoderma	<i>Staphylococcus</i> , <i>Streptococcus</i>	Often secondary to skin folds or self-trauma. Local cleansing and topical antibacterials adequate.		
	Malassezia dermatitis	<i>M. pachydermatis</i>	Identify, eliminate underlying causes. Use shampoos.	Ketoconazole	Itraconazole
	Dermatophytosis	<i>Microsporum</i> , <i>Trichophyton</i>	Topical and environmental measures needed as well. Localized lesions may not require systemic therapy.	Griseofulvin	Ketoconazole, itraconazole
	Bite wounds, traumatic and contaminated wounds	<i>Staphylococcus</i> , <i>Streptococcus</i> , <i>Pasteurella</i> , anaerobes	Wound irrigation and debridement most important. Parenteral antimicrobial might give effective prophylaxis within 3 hours of wounding, but of questionable value after 3 hours if appears uninfected. Treat empirically pending laboratory data if infected. Consider tetanus.	Penicillin G or V, isoxazolyl penicillin ^b	Amoxicillin-clavulanate, cephalosporin (oral, or group 1 parenteral) ^g
Ear	Anal sac inflammation, abscessation	<i>Escherichia</i> , <i>Enterococcus</i> , <i>Clostridium</i> , <i>Proteus</i>	Generally local treatment only. Systemic therapy warranted if inflammation severe, patient febrile.	Amoxicillin-clavulanate	Chloramphenicol
	Otitis externa	<i>Malassezia</i> , <i>Staphylococcus</i> , <i>Pseudomonas</i> , <i>Escherichia</i> , <i>Proteus</i>	Check for foreign material, hair, mites. Clean and dry canal. Initial empirical. Treatment often satisfactory, investigate if severe, persistent, or recurrent. Consider topical glucocorticoid or analgesic.	Topical aminoglycoside ⁹ or chloramphenicol	Topical nystatin, miconazole, or clotrimazole for <i>Malassezia</i> topical polymyxin ^h for Gram-negatives
	Otitis media, otitis interna	As for otitis externa	Otitis externa often present. Seek contributing causes. Clean and drain; use topical antimicrobials. Avoid ototoxic drugs (topical or systemic). Microbial investigation warranted.	Amoxicillin-clavulanate	Chloramphenicol, fluoroquinolone ⁱ ; ketoconazole or itraconazole, for <i>Malassezia</i>
Eye	Superficial ocular infection	<i>Staphylococcus</i> , <i>Streptococcus</i> , <i>Escherichia</i> , <i>Proteus</i>	Identify and correct contributing causes (poor eyelid conformation, hairs, dust, UV light, reduced tears).	Topical neomycin-polymyxin ^h -bacitracin	Topical chloramphenicol, gentamicin

(continued)

Table 29.2. Antimicrobial drug selection in canine infections.^a (continued)

Site or Type	Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs	Alternatives
	Penetrating eye wound	Various bacteria	Infection, uveitis possible. Sample and culture aqueous humor.	Amoxicillin - clavulanate with topical and episcleral gentamicin	Chloramphenicol (also topical) and episcleral chloramphenicol sodium succinate
Respiratory and thoracic	Bacterial rhinitis	Various resident species, usually secondary	Antibacterial drugs unlikely curative. Seek underlying cause (foreign body, allergy, fungus, tumor, virus, etc).	Amoxicillin ⁱ	Sulfonamide - trimethoprim ^f
	Fungal rhinitis	<i>Aspergillus</i> , <i>Penicillium rarer</i>	Exclude nasal neoplasia. May be secondary bacterial involvement (Chapter 19).	Topical clotrimazole	Topical enilconazole; systemic itraconazole or ketoconazole
	Infectious tracheo-bronchitis	<i>Bordetella</i> , viruses, <i>Mycoplasma</i>	Most recover untreated in 7-10 days, but antimicrobial therapy may hasten resolution. Secondary bacterial bronchopneumonia in some. Treat if systemically unwell.	Sulfonamide - trimethoprim ^f	Amoxicillin ⁱ (consider nebulized gentamicin if refractory)
	Bacterial pneumonia	Single (mostly) or mixed infection involving various facultative (especially Gram-negative) bacteria and anaerobes if aspirated	Aerobic culture and susceptibility test on material from transtracheal aspirate, bronchial wash or lavage, or fine needle lung aspirate; anaerobic culture if suspect aspiration.	Amoxicillin - clavulanate, sulfonamide-trimethoprim ^f	Beta-lactam ^k , with aminoglycoside ^l or fluoroquinolone ^l
	Pneumocystis pneumonia	<i>P. carinii</i>	Secondary to inherited or acquired immune deficiency.	Sulfonamide - trimethoprim ^f	Pentamidine
	Pyothorax, purulent pleuritis	Various and often mixed, involving anaerobes (including <i>Actinomyces</i>) alone or in combination with aerobic-facultative bacteria (<i>Nocardia</i> , <i>Corynebacterium</i> , <i>Pasteurella</i> , <i>Staphylococcus</i> , <i>Streptococcus</i>)	Identification and susceptibility test on aerobic-facultative organisms advisable. Drainage and lavage are important.	Penicillin G ^m , amoxicillin ⁱ , amoxicillin-clavulanate	Lincosamide ^c , chloramphenicol; for <i>Nocardia</i> , sulfonamide ⁿ + trimethoprim ^f
Alimentary and abdominal	Periodontitis, gingivitis	Resident anaerobic and facultative bacteria	Teeth cleaning, scaling, other dental treatment may be needed.	Penicillin G ^m , amoxicillin ⁱ	Metronidazole ± spiramycin, tetracycline ^o , clindamycin
	Malar or carnassial abscess	Resident oral flora	Extract tooth, curette alveolus, ensure drainage.	Penicillin G ^m , amoxicillin ⁱ	Amoxicillin - clavulanate, clindamycin
	Ulcerative gingivostomatitis	Anaerobes and other resident bacteria	Possible underlying immunodeficiency, metabolic or physical disorder. Use local treatments, possibly prolonged or intermittent systemic drugs. Consider glucocorticoid.	Penicillin G ^m , amoxicillin ⁱ	Metronidazole ± spiramycin, clindamycin

Table 29.2. Antimicrobial drug selection in canine infections.^a (continued)

Site or Type	Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs	Alternatives
	Tonsillitis, pharyngitis	Resident oropharyngeal flora	Often secondary to vomiting, regurgitation cough, oral inflammation.	Amoxicillin ⁱ , amoxicillin-clavulanate	Sulfonamide-trimethoprim ^f , tetracycline ^o
	Gastric helicobacteriosis	<i>Helicobacter</i>	Relation between infection and illness often unclear. Value of treatment unknown.	Amoxicillin and metronidazole (and famotidine)	
	Bacterial enterocolitis	<i>Salmonella</i>	Often subclinical, sometimes significant but self-limiting. Drug indicated if systemically ill but may prolong carrier state.	Fluoroquinolone ⁱ	Sulfonamide - trimethoprim ^f
		<i>Campylobacter</i>	Possible resident, or primary or secondary pathogen. Don't treat unless signs evident.	Macrolide ^d	Chloramphenicol, tetracycline ^o
		<i>Escherichia</i> , <i>Clostridium</i> , <i>Staphylococcus</i> , others	Normal residents, pathogenic role uncertain. Drug use questionable unless septicemia suspected.	Amoxicillin ⁱ , amoxicillin-clavulanate	Chloramphenicol, beta-lactam ^k with aminoglycoside ^l
	Giardiasis	<i>Giardia</i>	Infection often subclinical. Potential zoonosis.	Fenbendazole	Albendazole, metronidazole, tinidazole ^p
	Coccidiosis	<i>Isospora</i>	Infection probably irrelevant in adults or if few oocysts present. Often self-limiting.	Sulfonamide ⁿ ± trimethoprim ^f or pyrimethamine	Furazolidone, amprolium
	Parvoviral enteritis	Secondary facultative and anaerobic bacteria	Fluid therapy imperative. Consider antimicrobial if severe and potential septicemia. Parenteral route best.	Amoxicillin ⁱ , amoxicillin-clavulanate	Beta-lactam ^k , with aminoglycoside ^l or fluoroquinolone ⁱ
	Small intestine bacterial overgrowth	<i>Escherichia</i> , <i>Enterococcus</i> , <i>Staphylococcus</i> , <i>Clostridium</i>	Secondary to abnormal motility or anatomy, enteritis, maldigestion impaired immunity. Investigation, dietary therapy required. Drug choice empirical, may need continuous or intermittent dosage.	Amoxicillin ⁱ , amoxicillin-clavulanate, tetracycline ^o	Metronidazole, lincosamide ^e , chloramphenicol
	Colitis	Secondary infection by resident anaerobes and facultative bacteria	Bacteria irrelevant or secondary to stress, trichuriasis, immunodeficiency, viral or idiopathic disease. Dietary therapy, sulfasalazine suggested. Consider anti-anaerobe drug if unsuccessful or if acute colitis with systemic signs.	Metronidazole, chloramphenicol	Macrolide ^d , amoxicillin ⁱ
	Cholecystitis, cholangitis	<i>Escherichia</i> , <i>Salmonella</i> , anaerobes	May need surgery to restore bile flow, appropriate fluid and dietary therapy.	Amoxicillin-clavulanate	Beta-lactam ^k , with aminoglycoside ^l or fluoroquinolone ⁱ (continued)

Table 29.2. Antimicrobial drug selection in canine infections.^a (continued)

Site or Type	Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs	Alternatives
Urinary and genital	Intra-abdominal sepsis (bacterial peritonitis, abscessation)	Mixed anaerobes and facultative Gram-negative bacteria	Exploration, drainage, lavage (including antimicrobial drugs) may be needed. Culture and testing essential.	Gentamicin or fluoroquinolone ^l with one of amoxicillin ⁱ , clindamycin, metronidazole	Amoxicillin-clavulanate, chloramphenicol
	Pancreatitis	Enteric bacteria	Nursing and supportive treatment important.	Cefotaxime, chloramphenicol	Metronidazole, lincosamide ^c
	Lower urinary tract infection, cystitis	<i>Escherichia</i> , <i>Proteus</i> , <i>Staphylococcus</i> , <i>Streptococcus</i> , <i>Klebsiella</i> , <i>Pseudomonas</i> , <i>Enterobacter</i>	Usually single isolate. Seek contributing cause (calculi, tumor, urine retention, incontinence) especially if mixed infection. Culture cystocentesis sample and test susceptibility if no response or recurs (see Chapter 22).	Amoxicillin ⁱ , amoxicillin-clavulanate, sulfonamide-trimethoprim ^e	Tetracycline ^o , fluoroquinolone ^l , cephalixin
	Pyelonephritis	Similar to lower urinary infection, especially <i>Escherichia</i> , <i>Proteus</i> , <i>Staphylococcus</i>	Urine culture advised. Prolonged treatment, monitor response (see Chapter 22).	Amoxicillin ⁱ , amoxicillin-clavulanate	Fluoroquinolone ^l , sulfonamide-trimethoprim ^f
	Prostatitis	As for lower urinary infection	Often associated with urinary infection. Treat >3 weeks if acute, 6 weeks if chronic. Surgery for prostate abscess. Castration or progestin for prostatomegaly.	Sulfonamide - trimethoprim ^f , chloramphenicol	Macrolide ^d or lincosamide ^c if Gram-positive, fluoroquinolone ^l if Gram-negative
	Orchitis, epididymitis	<i>Escherichia</i> , <i>Brucella</i>	May be associated with urinary infection. Culture semen, urine, or needle aspirate. Consider castration.	Amoxicillin-clavulanate, sulfonamide-trimethoprim ^f	Chloramphenicol; doxycycline plus gentamicin for <i>Brucella</i>
	Balanoposthitis, vaginitis	Resident bacteria, herpesvirus (prepuce) <i>Mycoplasma</i> (vagina)	Look for predisposing factors. Local cleansing and antibacterial wash sufficient.		
	Metritis, pyometra	<i>Escherichia</i> , <i>Streptococcus</i> , <i>Staphylococcus</i> , other facultative anaerobes, possibly anaerobes	Ovariohysterectomy recommended. Prostaglandin plus antibiotic may be effective in open pyometra or metritis, but culture advisable.	Amoxicillin-clavulanate, chloramphenicol	Amoxicillin ⁱ , with aminoglycoside ^l or fluoroquinolone ^l
	Mastitis	<i>Escherichia</i> , <i>Staphylococcus</i> , <i>Streptococcus</i>	Check milk pH (affects penetration of systemic antimicrobials). Do Gram stain or culture and test susceptibility (see Chapter 30).	Chloramphenicol (pH unimportant) if weaning possible, otherwise amoxicillin-clavulanate	If pH >7.4: amoxicillin ⁱ , amoxicillin-clavulanate. If pH <7.3: sulfonamide-trimethoprim ^e for Gram-negatives, macrolide ^d for Gram-positives
	Local bacterial myositis	<i>Staphylococcus</i> , <i>Clostridium</i> , other anaerobes and facultative bacteria	Flora vary with cause (trauma, bite, surgery). Drainage, culture, susceptibility test best.	Amoxicillin - clavulanate	Chloramphenicol, cephalosporin (oral, or group 1 parenteral) ^f
Musculo-skeletal	Osteomyelitis	Mostly <i>Staphylococcus</i> , also <i>Streptococcus</i> , <i>Escherichia</i> , <i>Proteus</i> , <i>Pseudomonas</i> , <i>Klebsiella</i> , anaerobes	Laboratory evaluation advised. Promote drainage, remove foreign material, treat long-term (see Chapter 22).	Isoxazolyl penicillin ^b , amoxicillin - clavulanate	Lincosamide ^c , isoxazolyl penicillin ^b with aminoglycoside ^l or fluoroquinolone ^l

Table 29.2. Antimicrobial drug selection in canine infections.^a (continued)

Site or Type	Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs	Alternatives
Nervous system	Diskospondylitis, vertebral osteomyelitis	<i>Staphylococcus</i> ; also <i>Streptococcus</i> , <i>Brucella</i> , <i>Escherichia</i> , <i>Aspergillus</i>	Evaluate <i>B. canis</i> titer, culture blood, urine and aspirate from site to aid identification. Prolonged therapy. Curette if poor response (see Chapter 22).	Isoxazolyl penicillin ^b , amoxicillin - clavulanate	Cephalosporin (oral or group 1 parenteral) ^e ; for <i>Brucella</i> , doxycycline with gentamicin
	Septic arthritis	<i>Staphylococcus</i> , <i>Streptococcus</i> , less often anaerobes, coliforms	Possibly septicemic: culture blood, urine, joint fluid. Drain by needle or arthrotomy; use lavage (see Chapter 22).	Isoxazolyl penicillin, ^b amoxicillin-clavulanate	Cephalosporin (oral or group parenteral) ^e
	Bacterial meningitis	<i>Staphylococcus</i> , <i>Pasteurella</i> , <i>Actinomyces</i> , <i>Nocardia</i> , sometimes anaerobes	CSF culture and susceptibility test advised (see Chapter 22).	Amoxicillin - clavulanate	Sulfonamide - trimethoprim ^f , chloramphenicol
	Cryptococcal meningoencephalitis	<i>C. neoformans</i>	Guarded prognosis.	Amphotericin B with flucytosine or fluconazole	Amphotericin B, fluconazole, itraconazole
	Tetanus	<i>Clostridium tetani</i>	Use nursing, antitoxin, sedation, debridement, and inject penicillin G into wound area, too.	Penicillin G ^m	Tetracycline ^o , metronidazole
	Botulism	<i>Clostridium botulinum</i>	Supportive therapy mainly. Use antimicrobial only if gut colonized.	Penicillin G ^m	Metronidazole
Other bacterial	Hepatic encephalopathy	Normal intestinal flora	Oral antimicrobial drugs to suppress gut ammonia production; add low protein diet, lactulose.	Ampicillin	Neomycin
	Actinomycosis	Various <i>Actinomyces</i> spp.	Mostly with other bacteria in infections in subcutis, thorax, abdomen, retroperitoneum. Drainage, lavage, debridement, prolonged dosage needed.	Penicillin G ^m	Chloramphenicol, erythromycin, tetracycline ^o
	Bacteremia, bacterial endocarditis	Various Gram-positive and Gram-negative facultative bacteria, or anaerobic organisms, sometimes polymicrobial	Blood culture and susceptibility test preferred. Treat parenterally for 4-10 days then, if practicable and response satisfactory, use suitable oral therapy for 3-6 weeks.	Isoxazolyl penicillin ^b or amoxicillin ⁱ , with aminoglycoside ^l or fluoroquinolone ^j	Cephalosporin (parenteral group 1 or 2) ^e
	Bartonellosis	Several <i>Bartonella</i> spp.	Relation between infection and various diseases requires further investigation. May need 4-6 weeks' treatment to eliminate infection.	Azithromycin, macrolide ^d	Fluoroquinolone ^j ± amoxicillin ⁱ , doxycycline
	Brucellosis	<i>B. canis</i>	Potential zoonosis.	Doxycycline or minocycline, plus gentamicin	Other tetracycline ^o , with gentamicin or dihydrostreptomycin (continued)

Table 29.2. Antimicrobial drug selection in canine infections.^a (continued)

Site or Type	Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs	Alternatives
Other protozoal	Leptospirosis	<i>Leptospira</i> serovars <i>canicola</i> , <i>copenhageni</i> , <i>icterohaemorrhagiae</i>	Fluid and supportive therapy essential.	Amoxicillin ^l	Penicillin G ^m , tetracycline ^o , aminoglycoside ^l (streptomycin drug of choice for renal carrier)
	Lyme borreliosis	<i>Borrelia burgdorferi</i>	Aspirin or other non-steroidal anti-inflammatory for analgesia.	Doxycycline	Erythromycin, clarithromycin, amoxicillin ^l
	Nocardiosis	<i>N. asteroides</i>	Pulmonary, systemic or solitary extrapulmonary lesions.	Sulfonamide ± trimethoprim ^f	Amikacin, minocycline, erythromycin
	Septicemia, neonatal	<i>Streptococcus</i> , <i>Escherichia</i> , <i>Staphylococcus</i> spp.		Amoxicillin-clavulanate, or parenteral sulbactam-ampicillin	Cephalosporin (oral or parenteral group 1 or 2) ^e
	Tuberculosis	<i>Mycobacterium tuberculosis</i> , <i>M. bovis</i>	Prolonged, combination drug therapy required. Potential risk to owner.	Rifampin with enrofloxacin and azithromycin	Rifampin with isoniazid and ethambutol
	Babesiosis	<i>B. canis</i>	Supportive therapy is very important, may need blood transfusion, intravenous fluid with bicarbonate.	Imidocarb	Diminazene, pentamidine, phenamidine, trypan blue
		<i>B. gibsoni</i>	As for <i>B. canis</i> .	Atovaquone and azithromycin	Imidocarb
	Cryptosporidiosis	<i>C. parvum</i>	Infection usually subclinical, self-limiting. Disease risk in immunocompromised animals.	Paromomycin	Azithromycin
	Hepatozoonosis	<i>H. americanum</i> , <i>H. canis</i>	Drugs may reduce parasitemia without influencing signs. Relapses common. Use non-steroidal anti-inflammatory for analgesia.	<i>H. americanum</i> acute: clindamycin + sulphonamide + trimethoprim + pyrimethamine; chronic: decoquinat; <i>H. canis</i> : imidocarb	<i>H. americanum</i> : imidocarb; <i>H. canis</i> : diminazene, toltrazuril
	Leishmaniasis	Several <i>Leishmania</i> spp.	Resistant, infection often persists or relapses. Prognosis poor if in renal failure.	Meglumine antimonate and/or allopurinol	Sodium stibogluconate
	Neosporosis	<i>N. caninum</i>	Effects of tissue damage may persist even if infection eliminated.	Clindamycin	Sulfonamide ⁿ plus pyrimethamine
	Toxoplasmosis	<i>T. gondii</i>	As for neosporosis.	Clindamycin	Sulfonamide ⁿ plus pyrimethamine, azithromycin
	Trypanosomiasis, African	<i>T. brucei</i> , <i>T. congolense</i>	Combination treatment better but relapses common.	Diminazene plus difluoromethylornithine	Diminazene alone
	Trypanosomiasis, American	<i>T. cruzi</i>	Public health risk. Euthanasia might be preferred.	Nifurtimox	Benznidazole

Table 29.2. Antimicrobial drug selection in canine infections.^a (continued)

Site or Type	Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs	Alternatives
Rickettsial, ehrlichial, and mycoplasmal infections	Rocky Mountain spotted fever	<i>Rickettsia rickettsii</i>	Early, and supportive, therapy important. Zoonosis.	Doxycycline	Chloramphenicol, fluoroquinolone ^f
	Ehrlichiosis, anaplasmosis	<i>E. canis</i> , <i>E. risticii</i> , <i>E. ewingii</i> , <i>A. phagocytophilum</i>	Nursing, fluid therapy. Consider blood transfusion, anabolic steroid in marrow failure, glucocorticoid if thrombocytopenia or polyarthropathy.	Doxycycline	Imidocarb, minocycline, chloramphenicol
	Hemoplasmosis (haemobartonellosis)	<i>Mycoplasma haemocanis</i>	Transfuse blood if anemia life-threatening. Consider glucocorticoid.	Doxycycline	Chloramphenicol, fluoroquinolone ^f
	Other mycoplasmal infections	Various <i>Mycoplasma</i> spp.	Possible role in respiratory, urinary and reproductive disorders, polyarthritis, colitis.	Tetracycline ^g , chloramphenicol	Macrolide ^d , fluoroquinolone ^f
Systemic mycoses (Chapter 19)	Aspergillosis, disseminated	<i>A. terreus</i> , <i>A. deflexus</i> , <i>A. flaviceps</i> , <i>A. fumigatus</i>	Therapy may produce remission, despite persisting infection in immunocompromised patients.	Itraconazole	Amphotericin B
	Blastomycosis	<i>B. dermatidis</i>	Common sites lung, lymph node, skin, eye, bone.	Itraconazole	Amphotericin B plus ketoconazole
	Coccidioidomycosis	<i>C. immitis</i>	Infects lung, lymph node, bone, skin, eye, etc.	Ketoconazole	Amphotericin B ± ketoconazole; fluconazole, itraconazole
	Cryptococcosis	<i>C. neoformans</i>	Usually involves CNS, eye, lung, often disseminated.	Amphotericin B and/or fluconazole	Itraconazole
	Histoplasmosis	<i>H. capsulatum</i>	Commonly disseminated; affects gut, lung, liver, spleen, associated lymph nodes.	Itraconazole ± amphotericin B	Fluconazole

Notes

^aThese selections reflect personal opinion based on review of the literature, discussion with colleagues, and clinical experience. They are intended to guide drug selection when laboratory data are lacking. Selection may change once culture and drug susceptibility test results are known. See Greene (2005) for additional information.

^bIsoxazolyl penicillins: oxacillin, cloxacillin, dicloxacillin, flucloxacillin (Chapter 7).

^cLincosamides: lincomycin, clindamycin. The latter may be preferred but is much more expensive (Chapter 11).

^dMacrolides: erythromycin, tylosin (Chapter 12).

^eCephalosporins and related drugs: oral (cefachlor, cefadroxil, cephalexin, cephadrine), group 1 parenteral (cefazolin, cephapirin, cephradine), group 2 parenteral (cefotaxime, ceftiofur). (Chapter 8).

^fSulfonamide-trimethoprim: various sulfonamides (sulfadiazine, sulfamethaxazole, sulfadoxine etc) combined with trimethoprim. Ormetoprim and baquilo-prim are alternatives to trimethoprim (Chapter 16).

^gAminoglycosides, topical: neomycin, framycetin (neomycin B), kanamycin, gentamicin (Chapter 13).

^hPolymyxins: polymyxin B or colistin (polymyxin E). (See Chapter 10).

ⁱFluoroquinolones: difloxacin, enrofloxacin, ibafloxacin, marbofloxacin, orbifloxacin (Chapter 17).

^jAmoxicillin: ampicillin or hetacillin may be substituted (Chapter 7).

^kBeta-lactams: penicillins, cephalosporins (Chapters 7 and 8).

^lAminoglycosides, systemic: amikacin, gentamicin, tobramycin (Chapter 13).

^mPenicillin G: penicillin V may be substituted for oral administration (Chapter 7).

ⁿSulfonamides: many available, e.g., sulfadimidine, sulfadiazine, sulfadimethoxine (Chapter 16).

^oTetracyclines: doxycycline, minocycline, oxytetracycline, tetracycline (Chapter 14).

^pTinidazole: efficacy likely but data limited.

Table 29.3. Antimicrobial drug selection in feline infections.^a

Site or Type	Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs	Alternatives
Skin and subcutis	Superficial pustular dermatitis	<i>Streptococcus</i> , <i>Pasteurella</i>	Systemic drugs often unnecessary, use local cleansing, antiseptics.	Penicillin G ^b	Amoxicillin ^c
	Folliculitis, furunculosis	<i>Staphylococcus</i> , <i>Streptococcus</i>	Prolonged therapy may be needed for deep or recurrent lesions.	Penicillin G ^b or (for staphs) lincosamide ^d	Amoxicillin-clavulanate
	Cutaneous cryptococcosis	<i>C. neoformans</i>	Surgical removal or debulking helpful.	Fluconazole	Itraconazole, ketoconazole plus flucytosine
	Cat fight abscess	Anaerobes, also <i>Rhodococcus</i> , <i>Pasteurella</i> , <i>Staphylococcus</i> , <i>Actinomyces</i>	Use hot packs and surgical drainage as necessary.	Penicillin G ^b	Amoxicillin ^c , chloramphenicol, lincosamide ^d
	Feline "leprosy"	<i>Mycobacterium lepraemurium</i> ?	Surgical removal preferred.	Clofazimine ^e	Rifampin ^e , dapsone ^e , clarithromycin
	Opportunistic mycobacterial infections	<i>M. fortuitum</i> , <i>M. chelonae</i> , <i>M. smegmatis</i> , <i>M. phlei</i>	Surgical excision, debridement needed. Identification and susceptibility test advised.	Clarithromycin, with rifampin or fluoroquinolone	Doxycycline
	Variant tuberculosis syndrome	<i>M. tuberculosis</i> , <i>M. bovis</i> variant	Draining skin nodules, local lymphadenopathy. Rare systemic spread.	Clarithromycin with rifampin and enrofloxacin	
Ear	Otitis	Likely secondary infection: <i>Malassezia</i> , <i>Staphylococcus</i> , <i>Pasteurella</i>	Check for <i>Otodectes</i> mites, polyps, foreign bodies. Clean and dry ear canal.	Topical aminoglycoside ^g , polymyxin ^h , or chloramphenicol	Topical nystatin, miconazole, clotrimazole for <i>Malassezia</i>
Eye	Conjunctivitis	Herpes or calicivirus, calicivirus, <i>Chlamydomydia</i> , <i>Mycoplasma</i> , secondary bacteria	<i>Chlamydomydia</i> , <i>Mycoplasma</i> may be found in conjunctival scrapings.	Doxycycline, plus topical tetracycline ⁱ	Topical chloramphenicol. Topical idoxuridine or trifluridine for early herpes
	Penetrating eye wound	Various bacteria	Infection, uveitis possible. Sample and culture aqueous humor.	Amoxicillin-clavulanate with topical, episcleral gentamicin	Chloramphenicol (also topical) and episcleral chloramphenicol sodium succinate
Respiratory and thoracic	Acute viral upper respiratory tract infection	Secondary bacteria: <i>Staphylococcus</i> , <i>Streptococcus</i> , <i>Pasteurella</i> , possibly <i>Mycoplasma</i>	Nursing and supportive care.	Penicillin G ^b	Amoxicillin ^c , macrolide ^m
	Chronic rhinitis-sinusitis	As above, plus anaerobes	Intermittent treatment may help control, but not cure, the condition.	Penicillin V	Amoxicillin ^c , sulfonamide-trimethoprim ^k , chloramphenicol
	Cryptococcal rhinitis-sinusitis	<i>C. neoformans</i>	May also have pulmonary granulomas.	Fluconazole, itraconazole	Ketoconazole plus flucytosine
	Aspiration pneumonia	Mixed anaerobes and facultative bacteria	Analysis of tracheal wash fluid useful; could treat on suspicion if aspiration occurs; can be chemical pneumonitis rather than infection.	Amoxicillin ^c , amoxicillin-clavulanate	Chloramphenicol

Table 29.3. Antimicrobial drug selection in feline infections.^a (continued)

Site or Type	Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs	Alternatives
Alimentary and abdominal	Other bacterial pneumonia	<i>Pasteurella</i> , <i>Bordetella</i> , other facultative bacteria, anaerobes	Culture and susceptibility testing of transtracheal aspirate, bronchial wash, or lung aspirate recommended.	Amoxicillin ^c , amoxicillin-clavulanate	Sulfonamide-trimethoprim ^k , chloramphenicol
	Pyothorax	Various anaerobes, (including <i>Actinomyces</i> , <i>Bacteroides</i>) and <i>Pasteurella</i>	Chest drainage and lavage needed. Gram-stained smears should be examined, exudate cultured.	Penicillin G ^b , amoxicillin ^c , amoxicillin-clavulanate	Lincosamide ^d , chloramphenicol
	Periodontitis and gingivitis	Mixed facultatives and anaerobes	Remove calculus, improve dental hygiene. If severe, consider antibiotic prior to dentistry.	Penicillin G ^b , amoxicillin ^c	Metronidazole ± spiramycin; clindamycin
	Acute ulcerative gingivostomatitis	Various resident bacteria including anaerobes	Infection primary or secondary? Seek underlying cause. Local and supportive treatment.	Penicillin G ^b , amoxicillin ^c	Metronidazole ± spiramycin; clindamycin
	Chronic proliferative stomatitis	Bacteria secondary	Possibly viral etiology, hypersensitivity. Antimicrobials non-curative. Biopsy, treat locally, consider systemic glucocorticoid, dental extractions.		
	Gastric helicobacteriosis	<i>Helicobacter</i> spp.	Relation of infection to illness, and value of treatment unclear.	Amoxicillin and metronidazole (plus famotidine)	
	Bacterial enteritis	<i>Campylobacter</i>	Isolation of doubtful significance. Treatment may be unwarranted.	Erythromycin	Fluoroquinolone, tetracycline ^l
		<i>Escherichia coli</i>	As for <i>Campylobacter</i> .	Amoxicillin ^c	Sulfonamide-trimethoprim ^k , tetracycline ^l
		<i>Salmonella</i>	Mainly immunodeficient cats or kittens in poor conditions. Use drug only if systemically ill, but may prolong carrier state.	Fluoroquinolone	Chloramphenicol, sulphonamide-trimethoprim ^k
	Giardiasis	<i>Giardia</i>	Inapparent carriers occur, disease rare. Potential zoonosis.	Fenbendazole	Metronidazole
	Coccidiosis	<i>Isospora</i> spp.	Coccidia may be coincidental to diarrhea, but neonates and immunosuppressed adults at risk.	Sulfonamide ^m , sulfonamide-trimethoprim ^k	Furazolidone
	Parvoviral enteritis (panleukopenia feline infectious enteritis)	Secondary bacteria, especially anaerobes	Treat parenterally. Fluid therapy, nursing and supportive care essential.	Penicillin G, amoxicillin ^c	Amoxicillin-clavulanate, amoxicillin ^c plus gentamicin
	Cholecystitis cholangiohepatitis	Coliforms, <i>Pasteurella</i> , anaerobes		Amoxicillin-clavulanate	Beta-lactam ⁿ plus fluoroquinolone ^f , chloramphenicol
					(continued)

Table 29.3. Antimicrobial drug selection in feline infections.^a (continued)

Site or Type	Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs	Alternatives
Nervous system	Meningitis	<i>Pasteurella</i> , <i>Staphylococcus</i> , <i>Streptococcus</i> , <i>Cryptococcus</i>	Isolation and susceptibility test recommended. Guarded prognosis.	Penicillin G, amoxicillin-clavulanate Amphotericin B and flucytosine, fluconazole Ampicillin	Chloramphenicol, sulfonamide-trimethoprim ^k Itraconazole Neomycin
Other bacterial	Hepatic encephalopathy	Normal intestinal flora	Use low protein diet, lactulose, administer drugs orally.		
	Bacterial lower urinary tract infection	<i>Escherichia</i> , <i>Streptococcus</i> , <i>Staphylococcus</i> , <i>Proteus</i>	Rare. May be secondary to idiopathic cystitis, calculus, tumor. Antibacterial inappropriate unless bacteriuria present.	Amoxicillin ^c , amoxicillin-clavulanate	Sulfonamide-trimethoprim ^k , chloramphenicol
	Bartonellosis	<i>B. henselae</i> , others	Generally subclinical. Consider treating (for 2-4 weeks) if pet maintained with immunocompromised owner, but efficacy uncertain.	Azithromycin	Doxycycline
	Osteomyelitis	Mostly <i>Staphylococcus</i> , sometimes <i>Streptococcus</i> , coliforms, anaerobes	Surgical intervention, susceptibility test advised.	Amoxicillin-clavulanate, isoxazolyl penicillin ^g	Cephalosporin (oral or group 1 parenteral) ^h
	Plague	<i>Yersinia pestis</i>	Human health risk. Eliminate fleas.	Aminoglycoside ^g	Doxycycline, chloramphenicol, fluoroquinolone ^f
Other protozoal	Tuberculosis	<i>Mycobacterium bovis</i>	Rare if bovine tuberculosis controlled. Prolonged therapy. Potential risk to owner.	Rifampin with enrofloxacin and azithromycin	Rifampin with isoniazid and ethambutol
	Babesiosis	<i>B. felis</i>	Hemolytic anemia, consider blood transfusion.	Primaquine	
	Cytauxzoonosis	<i>C. felis</i>	Often fatal despite nursing, fluids, drugs.	Imidocarb	Diminazene
	Toxoplasmosis	<i>T. gondii</i>	Clinical disease uncommon.	Clindamycin	Sulfonamide ^m plus pyrimethamine, azithromycin
Mycoplasmal and chlamydial infections	Feline infectious anemia	<i>Mycoplasma haemofelis</i>	May be secondary to immuno-suppressive virus. Consider glucocorticoid with antimicrobial if primary. Transfuse blood if necessary.	Doxycycline	Fluoroquinolone ^f
	Other mycoplasmal infections	<i>M. felis</i> , <i>M. gateae</i> , others	Possibly in conjunctivitis, pneumonia, arthritis, abortion, fetal death, abscesses.	Topical tetracycline ⁱ for conjunctivitis	Other sites: tetracycline ⁱ , chloramphenicol, macrolide ⁱ , fluoroquinolone ^f
	Chlamydial infections	<i>Chlamydia felis</i>	Mainly ocular infections, possibly rhinitis pneumonia, genital infections.	Doxycycline plus topical tetracycline ^k for conjunctivitis	Other sites: tetracycline ⁱ , chloramphenicol, macrolide ⁱ , fluoroquinolone ^f

Table 29.3. Antimicrobial drug selection in feline infections.^a (continued)

Notes

Various other bacterial, protozoal and fungal infections occur infrequently in cats. Consult Table 29.2 (canine infections) for possible treatments.

^aThese suggestions are derived from review of the literature, discussion with colleagues, and clinical experience. Laboratory data (Gram stain of exudate or aspirate, or culture and susceptibility tests) should be used to guide drug selection if available. See texts listed in bibliography for additional information.

^bPenicillin G: Penicillin V may be substituted for oral administration (Chapter 7).

^cAmoxicillin: Ampicillin or hetacillin may be substituted (Chapter 7).

^dLincosamides: lincomycin, clindamycin (Chapter 11).

^eClofazimine, rifampin, dapsone: limited data on efficacy.

^fFluoroquinolones: difloxacin, enrofloxacin, ibafloxacin, marbofloxacin, orbifloxacin (Chapter 17).

^gAminoglycosides, topical: neomycin, framycetin (neomycin B), kanamycin, gentamicin (Chapter 13).

^hPolymyxins: polymyxin B, colistin (polymyxin E) (Chapter 10).

ⁱTetracyclines, topical: tetracycline, oxytetracycline, chlortetracycline (Chapter 14).

^jMacrolides: erythromycin, tylosin (Chapter 12).

^kSulfonamide-trimethoprim: various sulfonamides (sulfadiazine, sulfamethoxazole, sulfadoxine, etc.) combined with trimethoprim. Ormetoprim and baquilo-prim are alternatives to trimethoprim (Chapter 16).

^lTetracyclines, systemic: doxycycline, minocycline, tetracycline, oxytetracycline (Chapter 14).

^mSulfonamides: many available, e.g., sulfadimidine, sulfadiazine, sulfadimethoxine (Chapter 16).

ⁿBeta-lactam: penicillins, cephalosporins (Chapters 7 and 8).

^oIsoxazolyl penicillins: cloxacillin, dicloxacillin, flucloxacillin, oxacillin (Chapter 7).

^pCephalosporins, oral (e.g., cefadroxil, cephalexin) and group 1 parenterals (e.g., cephazolin, cephadrine) (Chapter 8).

^qAminoglycosides, systemic: amikacin, dihydrostreptomycin, gentamicin, kanamycin, streptomycin, tobramycin (Chapter 13).

potential for development of acute blindness in cats treated with enrofloxacin, and the prevalence of hypersensitivity reactions in Doberman Pinschers treated with trimethoprim-sulfonamide combinations. Drug selection may also have to be modified in renal failure, liver failure, pregnancy, or neonatal patients (Table 29.4; Chapter 4).

Drug Formulations

Previous problems with dosage forms not adapted for use in dogs and cats are easing as more manufacturers introduce products specifically for veterinary use, and with the increased access to compounding pharmacies, in some areas at least. There remains a need, however, for more oral formulations that are easier to administer to difficult patients, and also for drug preparations with pharmacokinetic characteristics that allow extended dosage intervals (24 hours or more). Transdermal antimicrobial preparations may appear attractive, but cannot be recommended without supportive pharmacokinetic data, as the use of inadequate products may promote development of antimicrobial resistance.

Generic drug preparations are frequently favored in larger patients for reasons of cost. Less concern is expressed about veterinary generics than their human counterparts and there is little evidence of a problem

at present. However, the potential for differences in bioavailability exists. As these could affect drug efficacy and safety, vigilance is advisable when changing from one brand or formulation of a drug to another.

Route of Administration

The route of administration is sometimes dictated by the drug chosen. For example, if an aminoglycoside or amphotericin B is selected to treat a systemic infection, it must be given parenterally because enteral absorption is poor. More often, several routes of administration are possible and the one chosen may depend on the disease being treated, the likely duration of therapy, the temperament of the patient, and the capability of the owner to administer the drug preparation.

Parenteral Administration

Injections are not routinely needed for antimicrobial therapy in dogs and cats, and some clinicians rarely use them. However, parenteral administration can be valuable to initiate treatment in severe infections where rapid systemic delivery of high drug concentrations is important. Other indications include fractious, unconscious, or vomiting patients, those with mouth pain, or infections susceptible only to antimicrobials that must be given parenterally.

The intravenous (IV) route should be used if maxi-

Table 29.4. Antimicrobial drugs that are potentially hazardous in renal failure, liver failure, pregnancy, or neonates.

Renal Failure ^a	Liver Failure ^b	Pregnancy ^a	Neonates ^a
Aminoglycosides	Chloramphenicol	Aminoglycosides	Aminoglycosides
Amphotericin B	Clindamycin	Amphotericin B	Chloramphenicol
Chloramphenicol(cat)	Griseofulvin (cat)	Azithromycin	Fluoroquinolones
Clarithromycin	Ketoconazole	Chloramphenicol	Metronidazole
Flucytosine	Lincomycin	Fluconazole	Nalidixic acid
Fluoroquinolones	Macrolides	Flucytosine	Nitrofurantoin
Lincomycin	Metronidazole	Fluoroquinolones	Polymyxins
Nalidixic acid	Rifampin	Griseofulvin	Sulfonamides
Nitrofurantoin	Sulfonamides	Ketoconazole	Rifampin
Polymyxins	Sulfonamide-trimethoprim (dog)	Metronidazole	Tetracyclines
Sulfonamides	Tetracyclines	Nitrofurantoin	Trimethoprim
Sulfonamide-trimethoprim (cat)		Polymyxins	
Tetracyclines (except doxycycline)		Sulfonamides	
		Tetracyclines	
		Trimethoprim	

^aSee Chapter 4.^bThere is little information on effects of liver failure on antimicrobial drug therapy. Some listed agents are potentially hepatotoxic, others might accumulate to toxic levels in hepatopathy. In general, these warnings constitute relative rather than absolute contraindications.

mum plasma drug concentrations are desired immediately after dosing, as with life-threatening infections. IV use might also be preferable in shocked or hypotensive patients, as poor peripheral perfusion may impede drug absorption from other sites.

Intramuscular (IM) or subcutaneous (SC) administration is usually safer and satisfactory in less demanding circumstances. These routes give similar bioavailability with most antimicrobial preparations, but SC administration is easier and generally causes less pain. Many formulations recommended for IM use can be given to dogs and cats by SC injection, but unfamiliar preparations should be assessed in a few animals first to check for possible injection site reactions.

For IM injections, the lumbar longissimus muscle may be a better site than the thigh. The preferred location lies midway between iliac crest and last rib, and halfway between dorsal spinous processes and the lateral border of the muscle. Injection here is less likely to be intermuscular, is usually well tolerated, and avoids the risk of major nerve damage.

Oral Administration

Dosage by the oral route is adequate in most infections and is generally the best method for home treatment. However, a struggle over oral medication can be counterproductive and dangerous, because of the risk of aspiration pneumonia, especially with oily medicines in

cats. Individual dogs and many cats are difficult to dose with solid dosage forms and some owners find it easier to use liquid formulations. These may need to be reasonably palatable if a struggle is to be avoided. With doxycycline, non-solid formulations may be preferred to tablets to minimize risks of esophageal irritation and ulceration; alternatively tablets can be followed by a bolus of water administered by syringe. In hospitalised patients, naso-oesophageal and orogastric intubation and gastrostomy tubes can be used as alternatives.

Administration of liquids, powders, or crushed tablets mixed in food may be possible. Some patients reject medicated food, but may be fooled into swallowing morsels of food containing a tablet or capsule, if first offered unmedicated pieces.

With all forms of oral or enteral administration, the potential effect of ingesta on drug bioavailability should be considered.

Influence of Food on Systemic Availability of Drugs Given Orally

Drug-food interactions that affect drug absorption are common in human patients but often overlooked in veterinary medicine. The most frequent outcome is reduced or delayed absorption of the drug, although sometimes it is increased or unaffected. The mechanisms responsible are complex and involve food-

Table 29.5. Suggested oral administration in relation to feeding.

Better when Fasting ^a	Better with Food	Indifferent to Feeding
Azithromycin	Cefadroxil ^b	Cephalexin ^b
Cephadrine	Chloramphenicol palmitate ^d	Chloramphenicol capsules, tablets ^{b,d}
Most erythromycin preparations ^b	Doxycycline ^e	Chloramphenicol palmitate ^b
Most fluoroquinolones ^c	Griseofulvin	Clarithromycin ^b
Isoniazid	Ibafloxacin ^{b,d}	Clindamycin
Lincomycin	Itraconazole	Ethambutol
Most penicillins ^b	Ketoconazole	Fluconazole
Rifampin	Metronidazole ^e	Hetacillin
Most sulfonamides	Nitrofurantoin ^e	Spiramycin ^f
Most tetracyclines		

Source: Data are from human studies, except as indicated.

^aAbsorption of these drugs may be reduced or delayed by ingesta. Fasting means no food for 1-2 hours before and 1-2 hours after dosing.

^bCanine data.

^cEnrofloxacin availability is reduced by ingesta in dogs. Effects of ingesta on fluoroquinolones are generally mild, but absorption may be delayed slightly. Dairy foods (and products containing multivalent cations) should be avoided.

^dFeline data.

^eFood may reduce gut irritation without hindering absorption importantly.

^fHuman data. Porcine data indicate better when fasting.

induced changes in gut physiology and direct interactions between food components and drugs. The composition of the meal, the volume of fluid ingested, and specific formulation of the drug may affect the outcome. Because of these complexities, it is not possible to give conclusive recommendations that cover all situations. It is also difficult to assess the importance of drug-food interactions as studies comparing therapeutic efficacy under fasting and nonfasting conditions are lacking. However, it may be prudent to fast patients for 1-2 hours before and 1-2 hours after administration of agents for which absorption can be impaired substantially by food, such as most penicillins and tetracyclines other than doxycycline. An alternative would be to give a higher dose with food, but the increase required is difficult to predict. Some antimicrobial drugs can be given without regard to feeding, while others might be better given with food to improve absorption or reduce gastric irritation associated with dosage. Current suggestions are shown in Table 29.5.

Dosing Rate and Duration of Therapy

Conventional dosage regimens for antimicrobial drugs in dogs and cats are presented in Table 29.6. These should be regarded as guidelines only. The optimum dosage regimen will vary somewhat with the case, de-

pending on the susceptibility of the pathogen, ability of the drug to reach the infection site, and competence of the patient's defences. Higher or more frequent dosages may be required for relatively resistant pathogens or lesions in tissues where drug penetration is poor. Smaller doses may be satisfactory in lower urinary infections if the drug (or its active metabolites) become highly concentrated in urine during the excretory process.

It is usually apparent within 2 days or so whether treatment is having the desired effect. If the response is inadequate, the diagnosis and treatment regimen should be re-evaluated. In most cases, selection of a different drug is warranted, but increased dosage of the original agent could be considered if underdosing or poor tissue penetration is suspected.

Treatment duration of 4-5 days, up to a week, is generally adequate for the majority of acute uncomplicated bacterial infections in dogs and cats. One suggestion is to treat for a minimum of 3 days and to continue for 2 days after signs of infection have subsided. Treatment responses may be slower with chronic infections, and prolonged administration (4-6 weeks) is often needed because of existing tissue damage, impaired blood supply, and compromised local or systemic immunity. For systemic mycoses, treatment for several months is usually required.

Table 29.6. Conventional dosage regimens for antimicrobial drugs in dogs and cats.

Drug	Route	Dose (mg/kg except as indicated)	Dose Interval (h)	Comments and Cautions
Penicillins, narrow spectrum				See Chapter 7.
Cloxacillin	PO, IV, IM	12.5-25	8	PO; avoid ingesta.
Dicloxacillin	PO	25	4-6	Avoid ingesta
	IV, IM	25	6-8	
Flucloxacillin	PO	15	8	Avoid ingesta.
Methicillin	IV, IM	25-50	6	
Oxacillin	PO, IV, IM	15-25	8	PO; avoid ingesta.
Penicillin G, Na or K	IV, IM, SC	12.5-25 (20,000-40,000 IU/kg)	4-6	Increases plasma Na or K.
Penicillin G, procaine	IM, SC	20 (20,000 IU/kg)	12-24	Once daily adequate routinely.
Penicillin G, benzathine	IM	30 (40,000 IU/kg)	72-120	Highly susceptible pathogens only; very limited use.
Penicillin V	PO	10 (15,300 IU/kg)	8	Avoid ingesta.
Penicillins, wider spectrum				See Chapter 7.
Amoxicillin	PO	10-20	8-12	Ingesta have minor effect.
	IV, IM, SC	7	12	
Amoxicillin-clavulanate	PO	12.5-25 (combined)	8-12	Use the higher dose for serious or systemic infections.
	IM, SC	10-20 (combined)	24	Use the higher dose for serious or systemic infections.
Ampicillin	PO	20-30	8	Avoid ingesta.
	IV, IM, SC	10-20	8	
Ampicillin-sulbactam	IV, IM	20-50 (combined)	8	Alternative to injectable amoxicillin-clavulanate.
Carbenicillin	IV, IM, SC	30-50	6-8	Use the lower dose for lower urinary tract infection, higher (to 150mg/kg) for systemic effect.
Hetacillin	PO	20-30	8	Prodrug for ampicillin.
Piperacillin	IV, IM	25-50	8-12	
Ticarcillin	IV, IM, SC	20-50	6-8	See carbenicillin.
Cephalosporins and relatives				See Chapter 8 for explanation of generations and groups.
Oral				
Cefaclor	PO	10-15	8	May cause vomiting, diarrhea, increased liver enzymes.
Cefadroxil	PO	20-30	12	May cause vomiting, diarrhea. Ingesta increase but delay systemic availability.
Cephalexin	PO	20-40	8-12	May cause vomiting, diarrhea, anorexia, salivation.
Cephadrine	PO	20	6-8	May cause vomiting, anorexia, diarrhea. Avoid ingesta.
Group 1 parenteral				
Cephazolin	IV, IM, SC	20-30	6-8	May cause pain, phlebitis.
Cephapirin	IV, IM, SC	10-30	8	May cause pain, phlebitis.
Cephadrine	IV, IM, SC	10-25	6-8	May cause pain, phlebitis.
Group 2 parenteral				
Cefotaxime	IV, IM, SC	20-50	6-8	
Ceftiofur	SC	2-4 (dog)	12-24	Use lower dose once daily for lower urinary tract infection, higher dose for systemic infections.
Group 3 parenteral				
Cefoperazone	IV, IM, SC	20-30	8-12	
Group 4 parenteral				
Cefoxitin	IV, IM, SC	30	6-8	A cephamycin. May cause vomiting IV, pain IM or SC.
Cefotetan	IV, IM, SC	30	8-12	A cephamycin. May cause vomiting IV, pain IM or SC.

Table 29.6. Conventional dosage regimens for antimicrobial drugs in dogs and cats. (*continued*)

Drug	Route	Dose (mg/kg except as indicated)	Dose Interval (h)	Comments and Cautions
Aminoglycosides				See Chapter 13. Watch for renal, vestibular, or auditory toxicity, neuromuscular blockade, circulatory depression. Single daily dose may reduce toxicity without compromising efficacy. Avoid in pregnancy, neonates; monitor geriatric and neonatal patients closely. May be ototoxic if used topically in ears with ruptured eardrums.
Amikacin	IV, IM, SC	15-30	24	Reserve for bacterial infections resistant to other aminoglycosides.
Dihydrostreptomycin	PO	10-20	6	Rarely used systemically.
	IM, SC	20-30	24	
Gentamicin	IV, IM, SC	6.6	24	
Kanamycin	PO	10	6	
	IV, IM, SC	30	24	
Neomycin	PO	10	6	
Streptomycin	PO	20	6	Rarely used systemically.
	IM, SC	20-30	24	
Tobramycin	IV, IM, SC	3-6	24	Reserve for bacterial infections resistant to other aminoglycosides.
Fluoroquinolones				See Chapter 17. Use lower end of dose ranges for urinary infections, higher end for soft tissue infections, osteomyelitis, <i>Pseudomonas</i> . May cause gastrointestinal disturbances, excitement (avoid in seizure-prone animals), nephrotoxicity (crystal deposition in tubules in alkaline urine). Avoid in pregnancy and in neonatal and growing animals (cartilage lesions). Avoid or reduce dose in renal failure. Acute blindness reported in some cats given enrofloxacin (usually, but not always, at higher doses).
Difloxacin	PO	5-10	24	
Enrofloxacin	PO, IV, IM, SC	2.5-10 (dog)	12-24	
	PO, IV, IM, SC	5 (cat)	24	
Ibafloxacin	PO	15	24	
Marbofloxacin	PO	2-5	24	
Orbifloxacin	PO	2.5-7.5	24	
Tetracyclines				See Chapter 14. Oral route preferred; painful IM. Absorption from gut reduced by ingesta. Side effects: anorexia, vomiting, diarrhea, fever in cats, tooth discoloration. Avoid in pregnancy, neonates, renal and hepatic failure.
Chlortetracycline	PO	20	8	
Doxycycline	PO, IV	5-10	12	Give with food to reduce gut irritation. Can be used in renal failure.
Minocycline	PO, IV	5-10	12	
Oxytetracycline	PO	20	8	
	IV, IM	10	12	
Tetracycline	PO	20	8	
	IV, IM	10	12	
Lincosamides, macrolides, and azalides				See Chapters 11 and 12. IM may be painful. Vomiting and diarrhea can occur with oral administration.
Azithromycin	PO	10 (dog)	24	An azalide.
	PO	5 (cat)	24-48	
Clarithromycin	PO	5-10	12	A newer macrolide.
Clindamycin	PO, IV, IM, SC	5-10	8-12	
Erythromycin	PO	10-20	8-12	Some erythromycin esters may be hepatotoxic, therefore avoid in liver disease.

(*continued*)

Table 29.6. Conventional dosage regimens for antimicrobial drugs in dogs and cats. (*continued*)

Drug	Route	Dose (mg/kg except as indicated)	Dose Interval (h)	Comments and Cautions
Lincomycin	PO	10-20	8-12	Often combined with metronidazole at 12.5 mg/kg (principally for treating oral infections).
	IV, IM	10-20	12-24	
Spiramycin	PO	23.4	24	
Tylosin	PO	10-20	12	See Chapter 16. Generally safe, but occasional adverse effects. In dogs: dry eye, dermatoses, polyarthritis, fever, blood dyscrasias, ataxia. In cats: salivation, depression, disorientation, cytopenias, azotemic renal failure. Potential folate deficiency. Avoid if anemic or leukopenic, during pregnancy, and in neonates.
	IV, IM	5-10	12	
Sulfonamides and combinations				
Sulfadiazine	PO, IV	50-100 (double first dose in dog)	12	See Chapters 15 & 18.
Sulfadiazine-trimethoprim	PO, IV, IM, SC	30 (combined)	12-24	
Sulfadimethoxine	PO, IV, IM, SC	25 (double first dose in dog)	24	
Sulfadimethoxine-baquiloprim	PO	30 (combined)	48	
	SC	30 (combined)	72	
Sulfadimethoxine-ormetoprim	PO	27.5 (combined, double first dose)	24	See Chapters 15 & 18.
Sulfamethoxazole-trimethoprim	PO	30 (combined)	12-24	
"Triple sulfas" (trisulfapyrimidines)	PO, IV	50 (double first dose in dog)	12	
Miscellaneous antibacterials				Few adverse effects in dogs. Oral dose safe in cats for 3 weeks, higher (60-100 mg/kg/day) possible up to one week. Side effects: depression, inappetence, vomiting, diarrhea, reversible marrow suppression. Avoid in neonates, pregnancy, liver failure, cats with renal failure.
Chloramphenicol	PO	50 (dog)	8	
	PO	50 mg total (cat)	12	
	IV, IM, SC	50 (dog)	8	
	IV, IM, SC	20 (cat)	12	Excise or debulk lesions if possible. May need prolonged therapy.
Clofazimine	PO	4-8 (dog)	24	
Dapsone	PO	8 (cat)	24	Reduce to 0.3-0.6 mg/kg in dogs after 2 weeks. May cause vomiting, diarrhea, anorexia, hemolysis, leukopenia, thrombocytopenia, dermatosis.
	PO	1 (dog)	8	
	PO	50 mg total (cat)	12-24	
Ethambutol	PO	15 (dog)	24	Toxicity: optic neuritis, peripheral neuritis, anorexia, vomiting.
Isoniazid	PO	10-20 (dog max. 300 mg daily)	24	Potentially hepatotoxic, neurotoxic.
Metronidazole	PO, IV, SC	7.5-15	12	May cause anorexia, vomiting, neurologic signs. Avoid in early pregnancy. Potential carcinogen. See antiprotozoal dose elsewhere.
Rifampin	PO, IV, IM	10-20 (dog max. 600 mg daily)	12-24	Hepatotoxic; avoid in liver disease, pregnancy. Discolors tears and urine.
Tinidazole	PO	20 (dog)	12	For anaerobes. See antiprotozoal dose elsewhere.
	PO	15 (cat)	24	
Vancomycin	IV	10-20	6-12	Infuse slowly over 30-60 minutes.
Other antiprotozoals and antirickettsials				
Allopurinol	PO	15	12	For 26 weeks. Combine with meglumine antimonate. Maintenance treatment 20 mg/kg/day given 1 week per month.
Amprolium	PO	100-300 mg total (dog)	24	Maximum daily dose in pups: small breeds 100 mg, large breeds 200 mg. Duration 7 days. Administer in food, water or capsules. Toxic effects: anorexia, depression, thiamine deficiency, CNS signs.
	PO	60-100 mg total (cat)	24	

Table 29.6. Conventional dosage regimens for antimicrobial drugs in dogs and cats. (continued)

Drug	Route	Dose (mg/kg except as indicated)	Dose Interval (h)	Comments and Cautions
Atovaquone	PO	13.3	8	For 10 days, with fatty meal; combine with azithromycin.
Benznidazole	PO	5	24	For 2 months.
Clindamycin	PO, IV, IM, SC	10-20 (dog)	12	Use the higher dose in smaller dogs; treat toxoplasmosis 2-3 weeks.
	PO, IV, IM, SC	12.5-25 (cat)	12	
Difluoromethylornithine	PO	100 (dog)	8	For 6 days, with diminazene. May cause diarrhea, vomiting.
Diminazene aceturate	IM	3.5 or 7 (dog)	Once	The lower dog dose for babesiosis, higher for African trypanosomiasis; repeat in 2-4 weeks. For cytauxzoonosis repeat at 72-96 hours. Pain at injection site. May cause gastrointestinal, CNS signs.
	IM	2 (cat)	Once	
Fenbendazole	PO	50	24	For 3 days.
Furazolidone	PO	4-10	12	For 7 days.
Imidocarb dipropionate	IM, SC	5	Once	Can repeat in 14 days. Injection painful, nodule may form at site. Causes salivation, lacrimation, diarrhea, dyspnea, depression.
Meglumine antimonate	IV, SC	100 (dog)	24	For 3-4 weeks. Treat relapses similarly. Side effects: anorexia, vomiting, lethargy, myalgia. Possible cardiac, renal toxicity. Inflammation at injection site.
Metronidazole	PO	10-25	12-24	See "Miscellaneous antibacterials" for antibacterial dose, cautions.
Nifurtimox	PO	2-7 (dog)	6	Continue 3-5 months.
Paromomycin	PO	125-165	12	For 5 days. Repeat if needed.
Pentamidine isethionate	IM	16.5 (dog)	24	Repeat in 24 hours. Side effects: Pain, necrosis at injection site, hypotension, nausea, salivation, vomiting, diarrhea, anaphylaxis.
Phenamidine isethionate	SC	15 (dog)	24	Once, or repeat in 24 hours. Side effects: see pentamidine.
Primaquine	PO, IM	0.5	Once	Single dose >1 mg/kg is lethal in cats.
Pyrimethamine	PO	0.25-0.5	12	Plus sulfonamide 30 mg/kg q12h for 2 weeks. Inappetence, depression, and malaise common in cats. Give folic acid 5 mg/day to reduce marrow suppression.
Sodium stibogluconate	IV, SC	30-50 (dog)	24	For 3-4 weeks. See meglumine antimonate, which has fewer side effects.
Tinidazole	PO	44 (dog)	24	For 3 days. See "Miscellaneous antibacterials" for antibacterial dose.
Toltrazuril	PO	5	12	For 5 days.
Trypan blue	IV	10	Once	Slow infusion 1% solution.
Antifungals				See Chapter 19.
Amphotericin B deoxycholate	IV, SC	0.25-0.5 (dog)	48	Infuse IV or inject SC in large volume. Maximum cumulative IV dose as single agent 8-12 mg/kg in dogs, 4 mg/kg in cats, higher SC (8-26 mg/kg).
	IV, SC	0.1-0.25 (cat)	48	Nephrotoxic.
Clotrimazole	Topical	1 g in 100 ml polyethylene glycol 400	Once	Slow infusion bilaterally. See package insert. Repeat in 4 weeks if persists.
Enilconazole	Topical	10	12	Dilute the 10% solution 50:50 with water just before irrigating nostrils, sinuses. Use 7-10 days.

(continued)

Table 29.6. Conventional dosage regimens for antimicrobial drugs in dogs and cats. (continued)

Drug	Route	Dose (mg/kg except as indicated)	Dose Interval (h)	Comments and Cautions
Fluconazole	PO	5-10 (dog)	24	Liver enzymes may increase with treatment.
	PO	50 mg total (cat)	8	
Flucytosine	PO	50-75 (dog)	8	Side effects: vomiting, diarrhea, leukopenia, thrombocytopenia, skin reactions. Combine with amphotericin B or an azole.
	PO	50 (cat)	8	
Griseofulvin microsize	PO	25-50	12	May cause gut upsets; and in cats, anemia, leukopenia, pruritis, ataxia, increased liver enzymes, bilirubinemia, teratogenicity. Administer for 6-10 weeks.
Griseofulvin ultramicrosize	PO	5-10	24	
Itraconazole	PO	5-10	12-24	May cause anorexia, increased liver enzymes and (in dogs) necrotic skin foci. Better tolerated than ketoconazole in cats.
Ketoconazole	PO	5-15 (dog)	12-24	May cause anorexia, depression, diarrhea, vomiting, coat changes, increased liver enzymes; in dogs reduced hormone production; in cats fever, anemia.
	PO	5-10 (cat)	24	Avoid in pregnancy.

Notes: Doses and intervals are similar for dogs and cats unless otherwise indicated. For additional dosage information, see Greene (2005).

Therapeutic Compliance

Carefully formulated therapeutic plans may be valueless if the owner does not follow the suggested dosage regimen. Problems may arise because the owner does not understand the importance of the medication or the instructions given. Furthermore, the owner's inexperience, patient's resistance, and suboptimum formulation characteristics (e.g., poor size, shape, taste, consistency) can prevent satisfactory administration and produce an angry animal and frustrated owner. These problems are likely to be greater with cats and some less congenial small dogs.

Issues of therapeutic noncompliance have not been well studied in veterinary medicine, but a few studies demonstrated poor compliance was common during treatment for acute bacterial infections in dogs. Potential difficulties should be addressed by scheduling dosing to suit the owner's routines. Linking dosage times to fixed points in the owner's day (e.g., mealtimes, bedtime) may assist, although the animal's mealtimes might need changing to avoid undesirable drug-food interactions. Other logical measures are to decide with the owners the dosage form they can best

manage, demonstrate its use, and provide clear verbal and written instructions. Human studies have shown that increasing treatment complexity is associated with increased probability that doses will be missed. Thus, if no therapeutic difference exists between two treatment options, the one with the less complex regimen should be prescribed. Likewise, additional medications of questionable value are best avoided, because ensuing complexity could reduce therapeutic compliance with the more important drugs.

Outcome

The response to antimicrobial therapy can be most favourable when the correct drug is used to treat an uncomplicated microbial infection in a patient that is otherwise healthy. By contrast, the outcome is likely to be disappointing if the wrong drug is chosen, if microbes are not responsible (or rather, viruses are), or if complicating factors have not been addressed. Additional specific and supportive measures, such as nursing, fluid therapy, and surgery, are often very important. If the response to appropriate therapy is poor or repeated relapses occur, an underlying maintaining

cause should be considered, including retroviral infections in cats, other immunoparetic disorders, tumors, and foreign bodies.

Bibliography

Ettinger SJ, Feldman, EC, eds. 2005. Textbook of Veterinary Internal Medicine, 6th ed. St Louis: Elsevier Saunders.

Greene CE, ed. 2005. Infectious Diseases of the Dog and Cat, 3rd ed. St Louis: Elsevier Saunders.

Maddison JE, Watson ADJ. 2002. Antimicrobial drugs. In: JE Maddison, SW Page, DB Church, eds. Small Animal Clinical Pharmacology. London: WB Saunders.

Nelson RW, Couto CG, eds. 2003. Small Animal Internal Medicine, 3rd ed. St Louis: Mosby.

Antimicrobial Drug Use in Cattle

Michael D. Apley, Johann F. Coetzee

Antimicrobial options for cattle have dramatically changed since the 1970s. Novel properties of new drug groups, changes in routes of administration, and advances in drug formulations have altered the characteristics of treatment regimens. Many of the newer antimicrobial therapies used in cattle have single-injection regimens. These new regimens are used in an environment of increased regulatory and political pressure, an expanding array of branded food product lines, and increased scrutiny by consumer and anti-animal-agriculture special interest groups. This chapter addresses important areas of consideration in constructing antimicrobial regimens in cattle within this context, including reasonable antimicrobials for selected diseases and extended discussions of some common therapeutic challenges.

General Considerations of Antimicrobial Use in Cattle

When giving treatment instructions to clients, especially in large-scale production facilities where lay personnel will be identifying and treating ill animals, the veterinarian is obligated to provide written treatment guidelines. The treatment guidelines should be constructed to contain the following information, where appropriate.

- Case definition for initial treatment
- Initial regimen
 - Drug(s), dose, route, frequency, duration, slaughter withdrawal
 - Specific administration instructions: injection site, volume per site, needle size, injection technique

- Environmental management during treatment: housing, water, feed
- Safety precautions or warnings
- Case definitions for treatment success and failure
- Secondary regimen for treatment of animals failing the initial treatment regimen
- Any additional regimens for animals not responding after the first and second regimens
- Disposition of animals not responding to therapy

It is essential that the treatment protocols not be altered without agreement by all parties involved. Consistency of protocol application is an absolute necessity in order to evaluate therapeutic and preventive programs in production systems.

In constructing these regimens, the veterinarian must make several key decisions.

One or multiple antimicrobials in each regimen.

The search for antimicrobial synergy is prevalent in all branches of medicine. However, there is little evidence that this is achieved in cattle. Anecdotal reports often claim that the preferred combination reduces relapses or improves initial treatment response. Arguments that combination therapy will suppress resistance development must be evaluated in light of considering that the bacterial population will also be exposed to a wider variety of antimicrobials.

Different therapy or continued therapy. If the animal did not respond to the initial antimicrobial regimen, was it because of pathogen resistance or the animal being incapable of responding in a short time? In the example of undifferentiated fever, which is often interpreted as respiratory disease in large pro-

duction systems, the animal typically has three to five days to respond to the initial regimen before being classified as a treatment failure. Newer antimicrobials give extended durations of antimicrobial coverage such as approximately seven days for ceftiofur crystalline-free acid (Excede®, Pfizer Animal Health), and 300 mg/ml long-acting oxytetracycline (Tetradure® 300, Merial), or up to approximately 14 days for tulathromycin (Draxxin®, Pfizer Animal Health). These longer durations of therapy bring forth the challenge of deciding when concentrations have reached a low enough level that non-responders should receive additional therapy.

It is reasonable to conclude, in the case of successful response by the majority of animals, that individuals not responding in a short time frame are in need of continued therapy as opposed to altered therapy. In the first author's personal experience with randomized, controlled respiratory disease trials, repeating the first regimen as continued therapy in first treatment failures resulted in second treatment response similar to those seen in trials where changes were made in therapy selections.

Quality assurance. The National Cattlemen's Beef Association beef quality assurance audits awoke the cattle industry in the United States to the need to carefully consider what is injected into cattle, and where it is injected. It is reasonable to give priority to antimicrobials with subcutaneous, intravenous, or oral administration routes. Current quality assurance guidelines call for injections in the neck when intramuscular administration is necessary. It is reasonable to avoid intramuscular injections of antimicrobials such as tylosin, erythromycin, and oxytetracycline. Injections should never go in the high-value muscles of the back, especially in the hip region, and should go in the hind leg only as a last resort.

Extra-label drug use (ELDU). In the United States, regulations for ELDU were promulgated as directed by the Animal Medicinal Drug Use Clarification Act (AMDUCA, 1996). The regulations should be consulted for actual guidance, but the overall order of expected use may be summarized as follows.

1. Use of an antimicrobial according to label directions.

2. Use of another food animal-labeled drug in an extra-label manner according to requirements set forth in the AMDUCA regulations.
3. Use of another veterinary labeled drug or human labeled drug according to requirements set forth in the AMDUCA regulations.
4. Use of a compounded product meeting the requirements of the AMDUCA regulations. It is well advised to consult the Food and Drug Administration/Center for Veterinary Medicine (FDA/CVM) compliance policy guideline on compounding.

Part of the AMDUCA regulation requirements is that the veterinarian must determine an extended slaughter withdrawal time for animals subjected to ELDU. In the United States, this information may be obtained from the Food Animal Residue Avoidance Databank (FARAD). If adequate information for construction of an extra-label slaughter withdrawal time is not available, then the drug may not be used in food animals. In some other countries this information is available through Global FARAD (gFARAD).

In the United States, the FDA/CVM has banned the following antimicrobials from extra-label use in food animals: chloramphenicol, fluoroquinolones, nitroimidazoles, nitrofurans, and glycopeptides. These regulations also prohibit the extra-label use of sulfonamides in lactating dairy cows (FDA/CVM, 2005). Veterinarians should be familiar with regulations in their countries in order to protect the interests of their clients and the consuming public.

Is Susceptibility Testing Useful in Selecting Antimicrobials for Use in Cattle?

The answer to this question depends on whether the susceptible and resistant breakpoints have been correlated to clinical efficacy. The Clinical Laboratory Standards Institute (CLSI), formerly the National Committee on Clinical Laboratory Standards (NCCLS), has approved veterinary-specific breakpoints for bovine respiratory disease (BRD) and mastitis for some antimicrobials (CLSI, 2003). These breakpoints have been established after reviewing pharmacokinetic, pharmacodynamic, and clinical trial data presented by the drug sponsor. Approved BRD-specific breakpoints have been established for ceftiofur

sodium, ceftiofur hydrochloride, ceftiofur crystalline free acid, danofloxacin, enrofloxacin, florfenicol, spectinomycin sulfate, and tilmicosin phosphate. Approved bovine mastitis breakpoints have been established for intramammary preparations of ceftiofur hydrochloride, penicillin/novobiocin, and pirlimycin. These breakpoints apply only when the antimicrobial is used according to label directions and the susceptibility testing is performed using CLSI approved methods and interpretive criteria.

For other antimicrobials, the breakpoints have been adapted from human interpretive criteria. Examples of this approach include penicillin G, the tetracyclines, potentiated sulfonamides, aminoglycosides, and erythromycin. It should be noted that there are no CLSI approved breakpoints for any antimicrobial agent, human or veterinary, for the treatment of enteric disease in any animal species. A more in-depth review of the use of susceptibility testing for bovine applications has been published (Apley, 2003).

Arguments that susceptibility testing results have no utility in antimicrobial selection are often based on the fact that animals with “susceptible” organisms have failed to resolve infections and animals with “resistant” pathogens have recovered. It is important to realize that antimicrobial susceptibility testing does not guarantee a specific clinical result in an individual animal. Rather, for those drugs with veterinary approved breakpoints, a susceptible result places the animal/drug regimen/pathogen combination in a population where clinical resolution is more likely, as opposed to those antimicrobial agents whose interpretive criteria were generated from human data. The veterinarian must determine when susceptibility testing may be of use in monitoring a population of animals and pathogens.

Judicious Use Guidelines

Concerns about the proper use of antimicrobials in food animals, especially related to resistance development in pathogens with zoonotic potential, have prompted veterinary specialty practice organizations to develop and publish judicious use guidelines. The American Veterinary Medical Association (AVMA) has made prudent use guidelines for the use of antimicrobials in cattle available on their website (AVMA, 2005). These guidelines were developed by the American Association of Bovine Practitioners (AABP) and were then approved by the AVMA Executive

Board. While these guidelines do not give specific recommendations for antimicrobial applications, they do provide overall guidance in the approach veterinarians should take in designing antimicrobial regimens for cattle.

Some antimicrobials have specific limitations in cattle that either preclude their use or require special consideration. The extra-label, systemic use of aminoglycosides in cattle has been the subject of resolutions or policy statements by the AVMA, AABP, Academy of Veterinary Consultants (AVC), and the National Cattlemen's Beef Association (NCBA). In general, these statements discourage the extra-label use of aminoglycosides in cattle due to the prolonged slaughter withdrawal potential. Veterinarians should pay special attention to these statements, especially when a producer organization joins with veterinary organizations in discouraging the extra-label use of a drug in cattle.

Some antimicrobials have significant potential for tissue damage when injected intramuscularly. These include the macrolides (tylosin, erythromycin) and the tetracyclines. Although as mentioned in the section on quality assurance, a visible lesion is not necessary for an adverse effect on tenderness, persistent visible lesions add to trim loss when primal cuts are fabricated into retail cuts. Intravenous use of tylosin and erythromycin are a possibility, but the non-water-soluble properties of these drugs in commercially available forms, combined with their propylene glycol carriers, make adverse reactions a possibility. In addition, repeated intravenous injections have become less attractive in light of effective alternatives with less frequent administration requirements.

Labeled antimicrobial applications and regimens for selected antimicrobials are reported in Table 30.1. While the regimens for labeled pathogens may not be optimal for extra-label use, these regimens provide a starting point for consideration. They also indicate a regimen where patient toxicity is not a concern and for which an adequate slaughter withdrawal has been determined by at least one regulatory agency.

Suggestions for specific therapeutic antimicrobial applications in cattle are reported in Table 30.2. Where appropriate, justifications for drug recommendations are provided in the table comments. Antimicrobial treatment of *Mycoplasma bovis*, enteric *Salmonella* spp., *E. coli*, and *Cryptosporidium parvum* are discussed below.

Table 30.1. Antimicrobial regimens for label indications.

Drug	Region Approval	For Use In	Indication(s)	Dose	Regimen
Amoxicillin	US	Cattle	Bovine respiratory disease, "shipping fever", pneumonia (<i>Mannheimia haemolytica</i> , <i>Pasteurella multocida</i> and <i>Histophilus somni</i>)	6.6 - 11 mg/kg	Q24h, 5 days max, IM or SC
	EU	Cattle, calves	Acute necrotic pododermatitis, "foot rot" (<i>Fusobacterium necrophorum</i>) <i>Actinobacillus lignieresii</i> , <i>Actinomyces bovis</i> , <i>Bacillus anthracis</i> , <i>Bordetella bronchiseptica</i> , <i>Clostridium</i> spp., <i>Corynebacterium</i> spp., <i>Escherichia coli</i> , <i>Fusobacterium</i> spp., <i>Histophilus</i> spp., <i>Mannheimia</i> spp., <i>Moraxella</i> spp., <i>Pasteurella</i> spp., <i>Proteus mirabilis</i> , <i>Salmonella</i> spp., <i>Staphylococci</i> and <i>Streptococci</i>	7 mg/kg	Q24h, 5 days IM
Amoxicillin/ clavulanic acid	EU	Cattle, calves	<i>Staphylococci</i> , <i>Streptococci</i> , <i>Corynebacteria</i> , <i>Clostridia</i> , <i>Bacillus anthracis</i> , <i>Actinomyces bovis</i> , <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Campylobacter</i> spp., <i>Klebsiella</i> spp., <i>Proteus</i> spp., <i>Pasteurella</i> spp., <i>Fusobacterium</i> <i>necrophorum</i> , <i>Bacteroides</i> , <i>Histophilus</i> spp., <i>Moraxella</i> spp. and <i>Actinobacillus lignieresii</i> . Respiratory infections Soft tissue infections (e.g. joint/navel ill, abscesses etc.) Metritis Mastitis	8.75 mg/kg (7 mg/kg amoxicillin and 1.75 mg/kg clavulanic acid)	Q24h for 3 to 5 days IM
Ampicillin trihydrate	US	Cattle and calves (including non- ruminating [veal] calves)	Respiratory tract infections, bacterial pneumonia, "shipping fever", calf pneumonia, and bovine pneumonia (<i>Aerobacter</i> spp., <i>Klebsiella</i> spp., <i>Staphylococcus</i> spp., <i>Pasteurella multocida</i> , <i>Escherichia coli</i>)	4.4 - 11 mg/kg	Q24h, 7 days max, IM
Ampicillin/ sulbactam	Canada	Cattle	Bacterial pneumonia, pasteurellosis, "shipping fever" complex caused by or complicated by bacteria resistant to ampicillin	3.3 (amp) and 6.6 (sul) mg/kg	Q24h, 3+ days, IM
Amprolium	US	Calves	Coccidiosis (<i>Eimeria bovis</i> , <i>Eimeria zuernii</i>)	Prevention: 5 mg/kg; treatment: 10 mg/kg	Prevention: 21 days; treat- ment: 5 days, PO in feed or water
Cephalexin	EU	Cattle	Metritis, foot rot, wounds, abscesses	7 mg/kg	Q24h, 5 days max, IM only
Cefquinome	EU	Cattle	Bovine respiratory disease, pneumonia (<i>Mannheimia haemolytica</i> , <i>Pasteurella multocida</i>) Acute bovine interdigital necrobacillosis, "foot rot", pododermatitis (<i>Fusobacterium necrophorum</i> , <i>Bacteroides melaninogenicus</i>) Septicemia in calves (<i>Escherichia coli</i>)	1 mg/kg; 2 mg/kg for sep- ticemia in calves	Q24h, 3-5 days, IM
Ceftiofur sodium	US	Cattle	Bovine respiratory disease, "shipping fever", pneumonia (<i>Mannheimia</i> <i>haemolytica</i> , <i>Pasteurella multocida</i> and <i>Histophilus somni</i>) Acute bovine interdigital necrobacillosis, "foot rot", pododermatitis (<i>Fusobacterium necrophorum</i> , <i>Bacteroides melaninogenicus</i>)	1.1 - 2.2 mg/kg	3 - 5 days, IM or SC

Table 30.1. Antimicrobial regimens for label indications.

Drug	Region Approval	For Use In	Indication(s)	Dose	Regimen
Ceftiofur hydrochloride	US	Cattle	Bovine respiratory disease, "shipping fever", pneumonia (<i>Mannheimia haemolytica</i> , <i>Pasteurella multocida</i> , <i>Histophilus somni</i>)	1.1 - 2.2 mg/kg	3 days; 4 - 5 days for BRD, footrot; 5 days for acute metritis; IM or SC
			Acute bovine interdigital necrobacillosis, "foot rot", pododermatitis (<i>Fusobacterium necrophorum</i> , <i>Bacteroides melaninogenicus</i>)		
			Acute metritis (0 to 14 days post-partum) associated with bacterial organisms susceptible to ceftiofur.		
Ceftiofur crystalline free acid	US	Cattle	Bovine respiratory disease, "shipping fever", pneumonia (<i>Mannheimia haemolytica</i> , <i>Pasteurella multocida</i> , <i>Histophilus somni</i>)	6.6 mg/kg	Once, SC in the ear
Chlortetracycline	US	Calves	Bacterial enteritis, "scours" (<i>Escherichia coli</i> , <i>Salmonella</i> spp.)	22 mg/kg/day; for diarrhea prevention: 1.1 mg/kg	5 days max, PO in water or PO in milk
			Bacterial pneumonia (<i>Pasteurella</i> spp., <i>Histophilus</i> spp., <i>Klebsiella</i> spp.)		
Danofloxacin	US	Cattle	Bovine respiratory disease, "shipping fever", pneumonia (<i>Mannheimia haemolytica</i> , <i>Pasteurella multocida</i>)	6 mg/kg	Twice 48 hrs apart, SC
Decoquinat	US	Cattle (including veal calves)	Coccidiosis prevention (<i>Eimeria bovis</i> , <i>Eimeria zuernii</i>)	0.5 mg/kg	At least 28 days, PO in feed and milk
Enrofloxacin	US	Cattle	Bovine respiratory disease, "shipping fever", pneumonia (<i>Mannheimia haemolytica</i> , <i>Pasteurella multocida</i> and <i>Histophilus somni</i>)	single dose: 7.5-12.5 mg/kg; multiple dose: 2.5-5 mg/kg	Multiple dose: 3-5 days, SC
Erythromycin	US	Cattle	Shipping fever, pneumonia, footrot, stress	1.1 - 2.2 mg/kg	Q24h, as needed, IM
Florfenicol	US	Cattle	Bovine respiratory disease (<i>Mannheimia haemolytica</i> , <i>Pasteurella multocida</i> , <i>Histophilus somni</i>)	20 mg/kg IM twice or 40 mg/kg SC once	
			Bovine interdigital phlegmon, "foot rot", acute interdigital necrobacillosis, infectious pododermatitis (<i>Fusobacterium necrophorum</i> , <i>Bacteroides melaninogenicus</i>)		
Gentamicin	US	Cattle	Infectious bovine keratoconjunctivitis, "pink eye" (<i>Moraxella bovis</i>)	0.75 mg	3 days max, ocular spray
Marbofloxacin	EU	Cattle, lactating dairy cattle	Pneumonia and shipping fever complex (<i>Pasteurella</i> spp., <i>Histophilus</i> spp)	2 mg/kg	Q24h for 3 to 5 days IM/IV/SQ
Monensin	US	Feedlot cattle, pasture cattle, mature reproducing beef cows, calves (excluding veal calves)	For the prevention and control of coccidiosis due to <i>Eimeria bovis</i> and <i>Eimeria zuernii</i> .	Feedlot cattle/veal calves: 0.14 to 0.42 mg/lb per day up to a maximum of 360 mg/day. Pasture cattle/ beef cows: same dose but not more than 100 mg/day for first 5 days.	Feed continuously
Neomycin	US	Cattle, not for use in veal calves	Colibacillosis, bacterial enteritis (<i>Escherichia coli</i>)	22 mg/kg/day	Up to 14 days, PO in water

(continued)

Table 30.1. Antimicrobial regimens for label indications.

Drug	Region Approval	For Use In	Indication(s)	Dose	Regimen
Neomycin/ oxytetracycline	US	Calves	Prevention and treatment of bacterial enteritis, "scours"	Doses vary greatly depending on label; values for prevention and treatment of disease reported.	PO in feed or milk replacer
Oxytetracycline (200 mg/ml)	US	Beef cattle, calves (including non-ruminating (veal) calves); bolus: not for use in veal calves	Pneumonia and shipping fever complex (<i>Pasteurella</i> spp., <i>Histophilus</i> spp.) Infectious bovine keratoconjunctivitis, "pinkeye" (<i>Moraxella bovis</i>) Foot rot and diphtheria (<i>Fusobacterium necrophorum</i>) Bacterial enteritis, "scours" (<i>Escherichia coli</i>) Wooden tongue (<i>Actinobacillus lignieresii</i>) Leptospirosis (<i>Leptospira pomona</i>) Wound infections, acute metritis (<i>Streptococcus</i> spp., <i>Staphylococcus</i> spp.)	20 mg/kg for pneumonia in calves and yearlings; 6.6 - 11 mg/kg for all indications; 11 mg/kg for footrot and advanced disease stages bolus.	20 mg/kg once; 6.6 - 11 mg/kg Q24h, max 4 days (IM, IM/SC, or SC depending on individual product label)
Oxytetracycline (300 mg/ml)	US	Cattle, (pre-ruminating) veal calves	Bovine respiratory disease, "shipping fever", pneumonia (<i>Mannheimia haemolytica</i> , <i>Pasteurella multocida</i> and <i>Histophilus somni</i>) Infectious bovine keratoconjunctivitis, "pinkeye" (<i>Moraxella bovis</i>) Acute bovine interdigital necrobacillosis, "foot rot", pododermatitis (<i>Fusobacterium necrophorum</i>) Bacterial enteritis, "scours" (<i>Escherichia coli</i>) Wooden tongue (<i>Actinobacillus lignieresii</i>) Leptospirosis (<i>Leptospira pomona</i>) Wound infections, acute metritis (<i>Streptococcus</i> spp., <i>Staphylococcus</i> spp.)	30 mg/kg, for BRD; 20-30 mg/kg for pink eye; 11 mg/kg for all others	Once, IM or SC for BRD and pink eye; Q24h, 4 days max, IM, IV, or SC
Penicillin G procaine	US	Cattle	Bacterial pneumonia, "shipping fever" (<i>Pasteurella multocida</i>)	6,600 IU/kg	Q24h, SC
Pen G procaine + benzathine combinations ("long-acting penicillin")	US	Cattle	Bacterial pneumonia, "shipping fever" (<i>Streptococcus</i> spp., <i>Arcanobacterium pyogenes</i> , <i>Staphylococcus aureus</i>) Upper respiratory infections, rhinitis or pharyngitis (<i>Arcanobacterium pyogenes</i>) Blackleg (<i>Clostridium chauvoei</i>)	4,400 IU/kg procaine pen + 4,400 IU/kg benzathine pen G	Twice, 48 hrs. apart, SC
Penicillin/ dihydrostreptomycin	EU	Cattle	Respiratory tract infections, listeriosis, septicemia, urogenital tract infections, enteritis	8 mg procaine penicillin and 10 mg dihydrostreptomycin sulphate per kg	Q24h, 3 days, IM
Spectinomycin	US	Cattle	Bovine respiratory disease, pneumonia (<i>Mannheimia haemolytica</i> , <i>Pasteurella multocida</i> , <i>Histophilus somni</i>)	10-15 mg/kg	3-5 days, SC

Table 30.1. Antimicrobial regimens for label indications.

Drug	Region Approval	For Use In	Indication(s)	Dose	Regimen
Streptomycin and/or dihydrostreptomycin	EU	Cattle	Wooden tongue (<i>Actinobacillus lignieresii</i>)	10 mg/kg	Q24h, 3 days, IM
Sulfachlorpyridazine	US	Calves under 1 month	Leptospirosis (<i>Leptospira pomona</i>) Diarrhea, colibacillosis (<i>Escherichia coli</i>)	66-99 mg/kg/day	Q12h for 1-5 days, PO or IV depending on label
Sulfadiazine/ trimethoprim	EU	Cattle	Urogenital tract infections (<i>Arcanobacterium pyogenes</i>); respiratory tract infections including rhinitis, pneumonia, bronchitis; pododermatitis	12.5 (sul) and 2.4 (trim) mg/kg	Once, or Q24h, 5 days max, IM or slow IV
Sulfamethazine	US	Ruminating replacement calves; sulfamethazine sodium drinking water solution: cattle	Bacterial pneumonia (<i>Pasteurella</i> spp.), colibacillosis, bacterial scours (<i>Escherichia coli</i>), calf diphtheria (<i>Fusobacterium necrophorum</i>). Some labels include coccidiosis (<i>Eimeria bovis</i> , <i>Eimeria zuernii</i>), acute metritis (<i>Streptococcus</i> spp.)	1 bolus/22.5 kg (363 mg/kg) or 1 bolus/91 kg or 0.1 g/4.5 kg first day, then 0.05 g/4.5 kg depending on label; drinking water solution: 247.5 mg/kg on day one then 135 mg/kg or 238 mg/kg on day one, then 119 mg/kg	Q72h, twice maximum OR Q24h max 5 days depending on label; drinking water solution: 4 days
Sulfamethazine/ chlortetracycline	US	Cattle	Respiratory disease, "shipping fever"	350 mg each of CTC and SMZ/day	28 days continuously, PO in feed
Sulfadimethoxine (injectable)	US	Cattle	Bovine respiratory disease complex, "shipping fever", bacterial pneumonia (<i>Pasteurella</i> spp.) Necrotic pododermatitis, "foot rot", calf diphtheria (<i>Fusobacterium necrophorum</i>)	55 mg/kg first dose, then 27.5 mg/kg	Q24h, IV
Sulfaquinoxaline	US	Cattle	Control and treatment of coccidiosis (<i>Eimeria bovis</i> , <i>Eimeria zuernii</i>)	6 mg/kg	3-5 days, PO in water
Tetracycline	US	Calves	Bacterial enteritis, "scours" (<i>Escherichia coli</i>) Bacterial pneumonia (<i>Pasteurella</i> spp., <i>Histophilus</i> spp., and <i>Klebsiella</i> spp.) "Shipping fever", hemorrhagic septicemia	22 mg/kg/day; soluble powder: for disease prevention: 100-200 mg/gallon; for treatment: 200-400 mg/gallon	2 - 5 days, PO or PO in water
Tilmicosin	US	Cattle	Bovine respiratory disease (<i>Mannheimia haemolytica</i>)	10 mg/kg	Once, SC
Tulathromycin	US, EU	Cattle	Treatment and control of bovine respiratory disease	2.5 mg/kg	Once, SC
Tylosin	US	Cattle	Bovine respiratory complex, "shipping fever", pneumonia, (<i>Pasteurella multocida</i> , <i>Arcanobacterium pyogenes</i>) Necrotic pododermatitis, "foot rot", diphtheria (<i>Fusobacterium necrophorum</i>) Metritis (<i>Arcanobacterium pyogenes</i>)	4 - 10 mg/kg	Q24h, 5 days max, IM

Table 30.2. Specific therapeutic antimicrobial application suggestions.

Label applications are United States labels except for those in **bold print**, which are European Union labels.

Category	Disease: Pathogen(s)	Drugs for which this disease is a label application (therapy and/or prevention)	Extra-label antimicrobials which are a reasonable choice	Unreasonable extra-label antimicrobial selections for this disease	Comments
Respiratory	Pneumonia: <i>Mannheimia</i> (<i>Pasteurella</i>) <i>haemolytica</i> , <i>Pasteurella multocida</i> , <i>Histophilus somni</i> (<i>Haemophilus somnus</i>)	Ampicillin trihydrate, <i>ceftiofur</i> (sodium, hydrochloride, and crystalline free acid salts), chlortetracycline, <i>danofloxacin</i> , <i>enrofloxacin</i> , <i>florfenicol</i> , oxytetracycline, chlortetracycline, procaine penicillin G (<i>P. multocida</i> only in the U.S.) spectinomycin sulfate, sulfadimethoxine, sulfamethazine, <i>tilmicosin</i> , <i>tulathromycin</i> , tylosin, cequinome , trimethoprim/sulfadiazine, trimethoprim/sulfadoxine, procaine penicillin/dihydrostreptomycin, amoxicillin trihydrate, amoxicillin/clavulanic acid		Gentamicin due to potential toxicity in dehydrated animals and prolonged renal residues in cattle	Antimicrobials with bovine respiratory disease on the label may be indicated for one or all of these pathogens. The italicized antimicrobials are the author's primary US choices for cattle in advanced stages of the disease or which have experienced extensive stress. Not all of the antimicrobials are labeled for all respiratory pathogens. The labels should be consulted for complete indications.
	Pneumonia: <i>Mycoplasma bovis</i>	Enrofloxacin , tylosin (<i>Mycoplasma</i> on label)	Oxytetracycline, florfenicol, tulathromycin, spectinomycin, fluoroquinolones*	Any beta-lactam (penicillins, cephalosporins) due to lack of a cell wall	See text for comments. *In the US, fluoroquinolones would only be legal when used for the purpose of respiratory disease due to the primary label pathogens.
	Diphtheria (necrotic laryngitis): <i>Fusobacterium necrophorum</i>	Oxytetracycline	Ampicillin, <i>ceftiofur</i> , penicillin G, sulfadimethoxine, tylosin		Extra-label recommendations are made based on published MIC values that are in the range of other pathogens successfully treated by these antimicrobials (Baba et al., 1989; Berg and Scanlan, 1982; Druan et al., 1991; Jousimies-Somer et al., 1996; Jang and Hirsh, 1994; Lechtenberg et al., 1998; Mateos et al., 1997; Piriz et al., 1990; Samitz et al., 1996). All of these isolates were from sites other than necrotic laryngitis. The nature of the site of necrotic laryngitis may make therapy with less lipid soluble antimicrobials more of a challenge.
Enteric	Scours, neonatal diarrhea: <i>E. coli</i>	Chlortetracycline, neomycin, oxytetracycline, sulfachlorpyridazine, sulfamethazine, tetracycline (all of these antimicrobials display consistently high MICs which suggest the drugs would be ineffective), amoxicillin/clavulanic	<i>Ceftiofur</i> , potentiated sulfonamides (all only after susceptibility testing)	These extra-label indications demonstrated very high MICs to most isolates: erythromycin, tylosin, <i>tilmicosin</i> , <i>lincomycin</i> , penicillin, ampicillin, florfenicol.	Recommended extra-label antimicrobials are based on susceptibility data and serum pharmacokinetics and should therefore be interpreted as relating to septicemia associated with enteric disease. See text for additional discussion.

Table 30.2. Specific therapeutic antimicrobial application suggestions. (continued)

Label applications are United States labels except for those in **bold print**, which are European Union labels.

Category	Disease: Pathogen(s)	Drugs for which this disease is a label application (therapy and/or prevention)	Extra-label antimicrobials which are a reasonable choice	Unreasonable extra-label antimicrobial selections for this disease	Comments
		acid bolus, cefquinome (septicemia), danofloxacin, enrofloxacin (septicemia and colibacillosis), marbofloxacin bolus, trimethoprim/ sulfadiazine, trimethoprim/sulfadoxine			
	Scours, neonatal diarrhea: <i>Salmonella</i> spp.	Chlortetracycline, oxytetracycline, (these antimicrobials display consistently high MICs which suggest the drugs would be ineffective), enrofloxacin , trimethoprim/sulfadiazine, trimethoprim/sulfadoxine, procaine penicillin/ dihydrostreptomycin	Ceftiofur, potentiated sulfonamides (all only after susceptibility testing)	Gentamicin will cause extended withdrawal times that will compromise the ability to slaughter an animal that recovers from the acute disease but does not return to satisfactory production.	Recommended extra-label antimicrobials are based on susceptibility data and serum pharmacokinetics and should therefore be interpreted as relating to septicemia associated with enteric disease. See text for additional discussion.
	Enterotoxemia, Overeating disease: <i>Clostridium perfringens</i> type C,D		Amoxicillin, ampicillin, penicillin G		Antiserum therapy is more likely related to therapeutic success. Septicemia resulting from enterotoxemia may involve multiple gut-related bacteria. Antimicrobial selection should reflect this possibility (see septicemia related to neonatal diarrhea above).
	Hemorrhagic bowel disease: <i>Clostridium perfringens</i> type A		Penicillin G, florfenicol		Prognosis of hemorrhagic bowel disease is very guarded, with surgery necessary for resolution in many cases (Dennison et al., 2002). There is no published evidence that antimicrobial intervention changes the clinical outcome. While there is no published data to support florfenicol efficacy in this disease, the general activity against anaerobes make it a reasonable consideration.
	Cryptosporidiosis: <i>Cryptosporidium parvum</i>	Halofuginone lactate (prevention, and reduction in excretion in affected calves)	For prevention: Lasalocid in calves \geq 1 week old (toxic in neonates at effective doses!)	Amprolium, sulfas	See text for comments on clinical trial data for cryptosporidiosis. Affected calves have severe acid/base and hydration insults.

(continued)

Table 30.2. Specific therapeutic antimicrobial application suggestions. (continued)

Label applications are United States labels except for those in **bold print**, which are European Union labels.

Category	Disease: Pathogen(s)	Drugs for which this disease is a label application (therapy and/or prevention)	Extra-label antimicrobials which are a reasonable choice	Unreasonable extra-label antimicrobial selections for this disease	Comments
	<i>Giardia</i>		Albendazole, fenbendazole, metronidazole (see comments)		The extra-label use of nitroimidazoles (e.g., metronidazole) in food animals is banned in the United States. Fenbendazole regimens of 5 mg/kg Q12H for 3 days or 5 mg/kg Q24H for 5 days, PO, have been suggested (Rings and Rings, 1996). Fenbendazole liquid is labeled for <i>Giardia</i> in puppies and kittens in the E.U.
	Coccidiosis: <i>Eimeria bovis</i> , <i>Eimeria Zuernii</i>	Prevention/control: monensin, lasalocid, amprolium, decoquinate, sulfaquinoxaline. Therapy of acute disease: sulfaquinoxaline, sulfamethazine, amprolium.	Sulfadimethoxine, sulfadimidine		
Genitourinary	Leptospirosis	Oxytetracycline, dihydrostreptomycin , tylosin (spirochetes on label)	Penicillin/dihydro-streptomycin, ceftiofur		Ceftiofur was effective in clearing induced leptospirosis (hardjo) in cows at 2.2 and 5.0 mg/kg Q24H for 5 days. These regimens were not effective when administered for 3 days. Long-acting 200 mg/ml oxytetracycline (20 mg/kg) and penicillin/ dihydrostreptomycin (25 mg/kg) were effective after single doses (Alt et al., 2001).
	Metritis/endometritis	Amoxicillin trihydrate , amoxicillin/clavulanic acid, cephalexin, ceftiofur hydrochloride, cephapirin (≥ 14 days postcalving), erythromycin, oxytetracycline 100, 200, and 300 mg/ml preparations, penicillin G (prophylaxis), sulfamethazine boluses, trimethoprim/sulfadiazine, trimethoprim/sulfadoxine, tylosin		Intrauterine administration of penicillins, aminoglycosides, and sulfonamides is questionable, as these may undergo enzymatic cleavage, operate poorly in an anaerobic environment, or lose activity in the presence of pus.	Chenault et al. (2004) reported 14-day cure rates of 77%, 65% and 62% for cows suffering from acute post-partum metritis treated with 2.2mg/kg IM/SQ ceftiofur HCl (CE) q24h for 5 d; 1.1 mg/kg CE q24h for 5 d, and controls, respectively. Königsson et al. (2000) demonstrated that cows treated with 10mg/kg IM oxytetracycline SID for 5 days demonstrated a shorter time to eradication of intrauterine <i>A. pyogenes</i> and <i>F. necrophorum</i> than untreated controls ($p < 0.05$).

Table 30.2. Specific therapeutic antimicrobial application suggestions. (*continued*)

Label applications are United States labels except for those in **bold print**, which are European Union labels.

Category	Disease: Pathogen(s)	Drugs for which this disease is a label application (therapy and/or prevention)	Extra-label antimicrobials which are a reasonable choice	Unreasonable extra-label antimicrobial selections for this disease	Comments
	Seminal vesiculitis: <i>Arcanobacterium pyogenes</i> , <i>Brucella abortus</i> , <i>E. coli</i> , <i>Pseudomonas</i> spp., <i>Actinobacillus seminis</i> , <i>Actinomyces bovis</i> , <i>Histophilus somni</i> , <i>Salmonella</i> spp., <i>Chlamydia</i> spp.		Antimicrobial therapy has not been shown to make a difference in clinical outcome. Oxytetracycline in the feed at various doses has been used for prevention. Tilmicosin phosphate, long-acting oxytetracycline, and florfenicol have been used in therapeutic attempts.		<i>Arcanobacterium pyogenes</i> is the most common agent in the US. <i>Brucella abortus</i> is the most common in countries with this disease. There is debate as to the role of bacterial or viral pathogens in the pathogenesis of seminal vesiculitis (Larson, 1997).
	Nephritis/ pyelonephritis: <i>Corynebacterium renale</i> , <i>Arcanobacterium pyogenes</i> , <i>E. coli</i>	Trimethoprim/sulfadiazine , trimethoprim/sulfadoxine	For <i>C. renale</i> , <i>Arcanobacterium pyogenes</i> - penicillin G, ampicillin. For <i>E. coli</i> - ceftiofur, fluoroquinolones (where legal).		
	Cystitis	Amoxicillin , trimethoprim/ sulfadiazine, amoxicillin trihydrate	Amoxicillin, ampicillin, ceftiofur, oxytetracycline, florfenicol, fluoroquinolones (where legal), penicillin G, trimethoprim/sulfa		Antimicrobials for cystitis have traditionally been chosen for their urine concentrations. However, the infection of concern is in the wall of the bladder, not the urine. Therefore, while urine concentrations may be of benefit, lack of significant urine concentrations does not necessarily preclude selection for cystitis.
Musculoskeletal	Adult arthritis - <i>Histophilus somni</i> , <i>Mycoplasma bovis</i>		Oxytetracycline, florfenicol, fluoroquinolones (where allowed by law), tulathromycin, spectinomycin, lincomycin (given due consideration to potential rumen flora alterations)	If <i>M. bovis</i> is suspected, any beta-lactam would be an unreasonable choice. If another organism is confirmed, then ceftiofur and ampicillin may be considered.	Other pathogens may be present as listed for neonatal arthritis. However, therapy of adult bovine arthritis should include consideration of these organisms unless ruled out by culture. Arthritis due to <i>M. bovis</i> is often characterized as a tenosynovitis. An extended duration of therapy (1-2 weeks) and a prolonged recovery period are necessary.

(*continued*)

Table 30.2. Specific therapeutic antimicrobial application suggestions. (continued)

Label applications are United States labels except for those in bold print , which are European Union labels.					
Category	Disease: Pathogen(s)	Drugs for which this disease is a label application (therapy and/or prevention)	Extra-label antimicrobials which are a reasonable choice	Unreasonable extra-label antimicrobial selections for this disease	Comments
	Neonatal arthritis: <i>E. coli</i> , <i>Arcanobacterium pyogenes</i> , <i>Staphylococcus</i> spp., <i>Streptococcus</i> spp.	Amoxicillin trihydrate, amoxicillin/clavulanic acid, procaine penicillin/dihydrostreptomycin, procaine penicillin G	Potentiated sulfonamides, Fluoroquinolones (where legal)		The potential presence of <i>E. coli</i> and the varied susceptibility results of ampicillin, florfenicol and oxytetracycline suggest they are not primary considerations for this disease. The primary metabolite of ceftiofur has a greatly elevated MIC ₉₀ value for <i>Staphylococcus</i> spp. as compared to the parent compound (Salmon et al., 1996), indicating it is not a primary choice where Staph spp. may be part of the infection.
Central nervous system	Listeriosis: <i>Listeria monocytogenes</i>	Procaine penicillin/ dihydrostreptomycin, procaine penicillin G	Penicillin G, oxytetracycline, enrofloxacin (depending on legal status). Therapy durations of 1-2 weeks may be necessary.		Varying results are reported for the recommended drugs. Five of six bulls in a case report survived after therapy with oxytetracycline and dexamethasone (Ayars et al., 1999). A sheep and goat case report indicated poor response to chloramphenicol and oxytetracycline, but six of nine animals recovered when treated with penicillin and gentamicin (Braun et al., 2002). Enrofloxacin has been reported as effective (Tripathi et al., 2001) but is illegal in countries with a ban on extra-label use of fluoroquinolones in food animals (e.g., United States).
	Thromboembolic meningoencephalitis (TME): <i>Histophilus somni</i>		Oxytetracycline, florfenicol		Oxytetracycline is a standard drug of choice for this application. Florfenicol is also suggested due to low MICs for <i>H. somni</i> combined with high lipid solubility.
	Meningitis: <i>E. coli</i> in neonates, multiple other pathogens possible		Ceftiofur, fluoroquinolones (where legal), trimethoprim/sulfa	Due to inconsistent coverage of the potential Enterobacteriaceae component: penicillin G, first-generation cephalosporins, macrolides, tetracyclines, florfenicol	While consideration of penetration of the blood-brain barrier is valid, it is likely that this barrier is disrupted in meningitis, allowing greater penetration of water-soluble compounds. Doxycycline is a lipid soluble tetracycline, but its high protein binding in serum limits the amount available to the diffusionary pool, and therefore CNS penetration.

Table 30.2. Specific therapeutic antimicrobial application suggestions. (*continued*)

Label applications are United States labels except for those in **bold print**, which are European Union labels.

Category	Disease: Pathogen(s)	Drugs for which this disease is a label application (therapy and/or prevention)	Extra-label antimicrobials which are a reasonable choice	Unreasonable extra-label antimicrobial selections for this disease	Comments
	Otitis media and interna: Potential pathogens include respiratory (all ages) and enteric pathogens (neonates). <i>Mycoplasma bovis</i> should be suspected in dairy calves where <i>M. bovis</i> mastitis is present in the herd.	Procaine penicillin/ dihydrostreptomycin Trimethoprim/sulfadiazine (infections of the ear on the label), tylosin	In cattle where respiratory pathogens are suspected: macrolides, florfenicol, fluoroquinolones (where legal). Beta-lactams might be expected to have lower concentrations in remote otic tissues.	Aminoglycosides may be expected to have extensive binding to protein debris at the site of infection and are less active in areas with lowered pH.	Without adequate trial data, extra-label recommendations are made on the basis of reported pathogen population MICs and lipid solubility of the compound. Many of the extra-label recommendations would have a hole in the spectrum for at least one possible pathogen (e.g., enrofloxacin - <i>Strep. spp.</i> , ceftiofur - <i>Staph. spp.</i> and <i>M. bovis</i> , macrolides and florfenicol - inconsistent against Enterobacteriaceae, penicillin G and ampicillin - Enterobacteriaceae and <i>M. bovis</i>).
Tissue/ integument	Infectious bovine keratoconjunctivitis (Pinkeye): <i>Moraxella bovis</i>	Oxytetracycline, topical gentamicin	Penicillin G, florfenicol, tilimicosin, topical benzathine cloxacillin		Florfenicol was found to be effective against IBK at either of the label dose regimens (Angelos et al., 2000; Dueger et al., 1999). Topical benzathine cloxacillin, 250 or 375 mg/eye, has been shown to be effective in naturally occurring and induced pinkeye models (Daigneault and George, 1990). Tilimicosin was shown to be effective at both 5 and 10 mg/kg (Zielinski et al., 1999). Although local penicillin G is a standard treatment, one report indicated no difference in healing of naturally occurring IBK after subconjunctival administration (Allen et al., 1995).
	Infectious pododermatitis (footrot): <i>Fusobacterium necrophorum</i> , <i>Bacteroides melaninogenicus</i>	Oxytetracycline, ceftiofur sodium and hydrochloride, tylosin, erythromycin, amoxicillin, sulfadimethoxine, sulfamethazine, cefquinome, tilimicosin, sulphadiazine/ trimethoprim	Procaine penicillin G, ampicillin trihydrate, florfenicol		Severe tissue reactions result from intramuscular use of tylosin and erythromycin.
	Actinobacillosis, "Wooden tongue" - <i>Actinobacillus lignieresii</i>	Long-acting oxytetracyclines (200 and 300 mg/ml), amoxicillin trihydrate, amoxicillin/clavulanic acid, cephalexin, dihydrostreptomycin, trimethoprim/sulfadiazine (Actinobacilli on label).	Streptomycin, sodium iodide combined with antimicrobial therapy for effect on granulomatous tissue.		A case report indicated that cattle receiving IV sodium iodide and intralesional streptomycin regressed lesions faster than negative controls or penicillin treated cattle (Campbell et al., 1975). No clinical trials are available.

(*continued*)

Table 30.2. Specific therapeutic antimicrobial application suggestions. (continued)

Label applications are United States labels except for those in **bold print**, which are European Union labels.

Category	Disease: Pathogen(s)	Drugs for which this disease is a label application (therapy and/or prevention)	Extra-label antimicrobials which are a reasonable choice	Unreasonable extra-label antimicrobial selections for this disease	Comments
	Actinomycosis, "Lumpy jaw" - <i>Actinomyces bovis</i>	Amoxicillin trihydrate, amoxicillin/clavulanic acid, dihydrostreptomycin, cephalexin, trimethoprim/sulfadiazine (Actinomyces on label)	Penicillin G, Ampicillin trihydrate, oxytetracycline. Sodium iodide may be combined with antimicrobial therapy for effect on granulomatous tissue.		No clinical trials are available to confirm efficacy of these antimicrobials. Prolonged therapy is recommended with surgical debridement of the lesion if possible.
	Blackleg: <i>C. chauvoei</i> ; Malignant edema: <i>C. sordellii</i> , <i>C. septicum</i> ; Tetanus: <i>Clostridium tetani</i> ; Bacillary hemoglobinuria: <i>Clostridium hemolyticum</i> ; Black's disease: <i>C. novyi</i> .	Amoxicillin trihydrate, amoxicillin/clavulanic acid, cephalexin, procaine penicillin G (<i>C. chauvoei</i>), procaine/benzathine penicillin G (<i>C. chauvoei</i>), tylosin	Penicillin G		All of the approved drugs have "clostridia" on the label, but most lack indications for specific clostridial diseases. Japanese isolates of <i>C. perfringens</i> , <i>C. septicum</i> and <i>C. sordellii</i> displayed phenotypic resistance to oxytetracycline and were confirmed to carry oxytetracycline resistance genes (Sasaki et al., 2001).
	Peritonitis: <i>E. coli</i> , <i>Aerobacterium pyogenes</i> , <i>Clostridium perfringens</i> , multiple Gram (+) and Gram (-) aerobes and anaerobes. Isolate reports in other species include organisms in all 4 quadrants.		Trimethoprim/sulfa (probably the most consistent for <i>E. coli</i>), florfenicol, oxytetracycline (both inconsistent on <i>E. coli</i>), ceftiofur for short withdrawal but may not cover <i>Staph. spp.</i>	Penicillin/gentamicin is reasonable as to spectrum but gentamicin engenders an extreme withdrawal that precludes salvage slaughter attempts in recovered animals.	No clinical trials are available in cattle. Recommendations are based on wide spectrum, lipid solubility, and duration of activity. An extended duration of therapy (≥ 1 week) is necessary. Prognosis is extremely poor in advanced cases. Note that the MIC ₉₀ of the ceftiofur metabolite against <i>Staph. spp.</i> is approximately 8 times that of the parent compound.
	Omphophlebitis (naval ill)	Amoxicillin trihydrate, amoxicillin/clavulanic acid, procaine penicillin/dihydrostreptomycin, procaine penicillin G,			
	Trichophytosis (Ringworm)	Benzalkonium chloride (0.15% topical solution), enilconazole, natamycin	Topical iodine solution/scrub, systemic griseofulvin*		*Regulations and availability of extra-label slaughter withdrawal time information should be confirmed prior to using griseofulvin in countries without a label for this application. Griseofulvin is teratogenic.

Table 30.2. Specific therapeutic antimicrobial application suggestions. (continued)

Label applications are United States labels except for those in **bold print**, which are European Union labels.

Category	Disease: Pathogen(s)	Drugs for which this disease is a label application (therapy and/or prevention)	Extra-label antimicrobials which are a reasonable choice	Unreasonable extra-label antimicrobial selections for this disease	Comments
	Rainrot (Dermatophilosis): <i>Dermatophilus congolensis</i>		Penicillin G, oxytetracycline		Penicillin G and oxytetracycline are often cited for therapy of dermatophilosis. A paper evaluating MIC and MBC concentrations, in vitro data, and unbound serum concentrations also recommended erythromycin, ampicillin, streptomycin, amoxicillin, and chloramphenicol (Hermoso de Mendoza et al., 1994). The chloramphenicol results suggest potential for florfenicol efficacy.
Cardiovascular/ systemic	Anaplasmosis	Chlortetracycline in the feed for control of active infection.	Oxytetracycline, imidocarb diprionate		Prevention or amelioration of clinical signs with oxytetracycline is well established. However, there are reports in the literature citing both successful and unsuccessful clearance of carriers with oxytetracycline. Recent work has documented unsuccessful clearance of induced anaplasmosis carrier status with the OIE regimen of 22 mg/kg oxytetracycline IV, Q24H, for 5 days. (Coetzee et al., 2005). Clearance of the carrier state with imidocarb has been documented (Roby and Mazzola, 1972).
	Endocarditis: <i>Arcanobacterium pyogenes</i> and <i>Streptococcus</i> spp. are most common. <i>E. coli</i> , other organisms also possible.		Penicillin G, presence of a Gram (-) on blood culture indicates ampicillin, amoxicillin, or ceftiofur.		Prolonged therapy is necessary. Addition of rifampin (5 mg/kg, PO, Q12H) has been suggested to improve response. Prolonged therapy (4-6 weeks) has been suggested. (Dowling and Tyler, 1994; McGuirk, 1991). Lack of clinical efficacy may be due to lack of antimicrobial penetration into vegetative lesions. Florfenicol would be appropriate for pathogens with appropriate MICs (variable on <i>E. coli</i>). In cases where the law and economics permit, fluoroquinolones would be appropriate if an organism other than a <i>Strep.</i> spp. was confirmed.

(continued)

Table 30.2. Specific therapeutic antimicrobial application suggestions. (*continued*)

Label applications are United States labels except for those in **bold print**, which are European Union labels.

Category	Disease: Pathogen(s)	Drugs for which this disease is a label application (therapy and/or prevention)	Extra-label antimicrobials which are a reasonable choice	Unreasonable extra-label antimicrobial selections for this disease	Comments
	Anthrax: <i>Bacillus anthracis</i>	Amoxicillin, amoxicillin/clavulanic acid, tylosin (<i>Bacillus</i> on label)	Penicillin G, oxytetracycline, fluoroquinolones (where legal) doxycycline, first generation cephalosporins. Chloramphenicol results suggest florfenicol may be an option.		A study evaluating the MICs of 25 genetically diverse <i>B. anthracis</i> isolates from multiple countries reported MIC ₉₀ values as follow: ciprofloxacin 0.09 µg/ml, penicillin 0.2 µg/ml, doxycycline 0.34 µg/ml, cefuroxime 32 µg/ml, cephalixin 0.25 µg/ml, cefaclor 1.65 µg/ml, and tobramycin 0.97 µg/ml (Coker et al., 2002). Except for cefuroxime, and possibly cefaclor, these MIC ₉₀ values are in a range where efficacy might be expected with typically used doses. Universally "susceptible" disk diffusion results with unvalidated interpretive criteria have been reported for tetracycline, ampicillin, streptomycin, chloramphenicol, and erythromycin in South African isolates (Odendaal et al., 1990).

Table 30.3. *Mycoplasma bovis* susceptibility data.

Antimicrobial	*S* breakpoint (µg/ml)*	Rosenbusch (2005) 223 US isolates			Ayling (2000) 62 British isolates			Draxxin™ Label (2005) 35 US isolates	
		MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)	Range (µg/ml)	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)	Range (µg/ml)	MIC ₉₀ (µg/ml)	Range (µg/ml)
Enrofloxacin	0.25	0.25	0.5	0.03 to 4	—	—	—	—	—
Danofloxacin	1	—	—	—	0.5	0.5	0.25 to 8	—	—
Florfenicol	2	1	4	0.06 to 8	4	16	4 to 128	—	—
Chlortetracycline	4	4	16	0.25 to >32	—	—	—	—	—
Oxytetracycline	4	2	16	0.125 to >32	32	64	2 to >128	—	—
Spectinomycin	32	2	4	1 to >16	4	>128	2 to >128	—	—
Tilmicosin	8	64	>128	0.5 to >128	>128	>128	16 to >128	—	—
Tulathromycin	N/A	—	—	—	—	—	—	1	≤0.063 to 2

*Breakpoint MIC used to return a "susceptible" finding for bovine respiratory disease (BRD) pathogens as designated by the Clinical Laboratory Standards Institute. This breakpoint has not been validated for BRD for chlortetracycline or oxytetracycline. At the time of publication, the danofloxacin breakpoint is sponsor-recommended only. None of these breakpoints have been validated for therapeutic success in disease involving *M. bovis*.

N/A: Not available at the time of publication.

Disease-Specific Discussions

Mycoplasma bovis

There is debate as to whether *M. bovis* is a primary respiratory pathogen in cattle. Standardized methods for MIC determination and interpretation of *M. bovis* susceptibility data have not been established. No clinical trials evaluating antimicrobial therapy of pneumonia or tenosynovitis due to *M. bovis* are available. Extra-label use recommendations by the author are made on the basis of published *M. bovis* susceptibility data indicating MICs in the range of other respiratory pathogens for which efficacy has been established (Ayling et al., 2000; Rosenbusch, 1998; Rosenbusch et al., 2005; Draxxin label, 2005). Selected MIC data is presented in Table 30.3.

Reasonable therapy strategies for *M. bovis* infections include the tetracyclines, spectinomycin, florfenicol, and tulathromycin. The available susceptibility data suggest there are refractive isolates for any of these drugs, so variation in therapeutic response is expected. It is also reasonable to consider that given the nature of the pathogen, therapy for *M. bovis* infections should consist of high antimicrobial concentrations for an extended period, possibly 10 days or longer.

While the fluoroquinolones show good in vitro activity against *M. bovis*, extra-label use of any of the fluoroquinolones in food animals is illegal in the US. Therefore, use against primary *M. bovis* disease in the

absence of the label BRD pathogens would be illegal in the US. In countries without this restriction, the fluoroquinolones show promise as a *M. bovis* primary treatment if adequate dose and duration of therapy is provided.

Enteric Disease and Septicemia Associated with *Escherichia coli* and *Salmonella* spp.

A review of recent literature confirms that there is a paucity of data on antimicrobial therapy for bacterial enteric disease in calves (Constable, 2004).

However, the authors' discussions with practitioners indicate that few would be willing to forego antimicrobial therapy, considering that a proportion of the calves are likely septicemic. Also, the potential for septicemia in adult cattle with coliform mastitis and salmonellosis calls for guidance in reasonable antimicrobial selection.

Tables 30.4, 30.5, and 30.6 describe in vitro antimicrobial susceptibility testing "results" using extended concentration ranges. Results are reported from Iowa State University diagnostic laboratory isolates (Hoffman and Klinefelter, 2005). While the history of exposure to antimicrobial agents for these organisms is unknown, these data are still of value in providing information as to what the overall pathogen antibiogram is for animals from which diagnostic samples are collected. However, because of the date and region-

ality of these data, they should not serve as a replacement for determining the susceptibility profile of your specific pathogen challenge.

There are some limitations and qualifications to understand before examining these data:

1. Shaded areas indicate the concentrations tested. Darker shading represents the susceptibility breakpoint for each drug: Inhibition of growth at or below this concentration would be reported as susceptible. (Note that these breakpoints have not been correlated to clinical response for these drugs and organisms. Laboratories may use different breakpoints.)
2. The value in the lowest concentration tested should be interpreted as less than or equal to that concentration because the actual MIC could be lower than the lowest value tested.
3. When the highest concentration tested did not inhibit growth of the organism, the result was reported as the next higher concentration. This would be the next concentration tested if the range was extended higher and could be interpreted as being greater than or equal to that value. In these cases, the actual MIC could be any value higher than the actual range tested.

4. A reported MIC indicates that the actual inhibitory value is somewhere between the reported value and the next lowest concentration.
5. Lower MICs for one drug compared to another drug do not necessarily equate to superior efficacy for the drug with the lower MICs.

Table 30.4 contains K99-positive *E. coli* bovine isolate susceptibility testing data for 2001-2003 from Iowa State University. The data for K99-negative isolates are not reported here, but are essentially the same. A biphasic population is evident when tested against trimethoprim/sulfa, ampicillin, gentamicin, and spectinomycin, with a clear majority of the isolates exhibiting MICs that would be considered resistant by breakpoints established for other diseases. Florfenicol has a few potentially susceptible isolates, but the majority appear to be resistant. Antimicrobials classically considered effective against *E. coli* (ceftiofur and enrofloxacin) display more evenly distributed biphasic distribution. Extra-label use of fluoroquinolones in food animals in the US is illegal, ruling out the use of enrofloxacin in enteric disease. Chlortetracycline, oxytetracycline, neomycin, and sulfas appear to be consistently poor choices. Benzyl penicillin,

Table 30.4. Iowa State University 2001-2003 bovine K99 positive *Escherichia coli* susceptibility testing results (n=146).

Drug	Number of Isolates Displaying Each Minimal Inhibitory Concentration (µg/ml)												
	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Ampicillin					10	3				133			
Apramycin						52	64	5			25		
Ceftiofur			69	2		12	40	23					
Chlortetracycline					1	2	4	139					
Clindamycin						146							
Enrofloxacin	85					61							
Erythromycin							146						
Florfenicol					4	39	5	98					
Gentamicin				51	6		4	85					
Neomycin						6			4		136		
Oxytetracycline				1	3			142					
Penicillin								146					
Sulfadimethoxine									2	4	4		136
Spectinomycin							4	25	8	1	108		
Sulfachlorpyridazine									10		6	12	118
Sulfathiazole									9	1			136
Tilmicosin								1	35	110			
Trimeth/sulfa			45	2	1	98							
Tylosin concentrations:					2.5	5	10	20	40				
Tylosin					1				145				

Shaded: concentrations tested. Dark shading: susceptibility breakpoint.

macrolides and lincosamides are drugs to which *E. coli* is intrinsically resistant. Because the concentration of drug used to treat enteric infections is different from that used to treat systemic infections, the application of these data should be restricted to systemic infection, as opposed to infections confined to the gut lumen.

Tables 30.5 and 30.6 summarize susceptibility testing data for *Salmonella* Dublin and *Salmonella* Newport bovine isolates from Iowa State University 2001–2003. Most of the *Salmonella* isolates in the ISU dataset display MICs at or above the highest concentration used for the majority of drugs tested. Exceptions for *S. Dublin*, with MICs often at the lowest concentrations tested, are apramycin, enrofloxacin, gentamicin, trimethoprim/sulfa, and ceftiofur. However, some isolates display high MICs to one or more of these drugs. Note that the *S. Newport* isolates have a different distribution for gentamicin and ceftiofur. There is potential therapeutic success with spectinomycin. Regulatory prohibition of extra-label use (US) and excessive withdrawal issues apply for fluoroquinolones and gentamicin respectively

when used for salmonellosis. Also in the US, extra-label use of sulfas is prohibited in lactating dairy cows (TMP/sulfa would be extra-label in this instance). With these factors in mind, Table 30.6 illustrates that for many *S. Newport* infected cows in the US, there are few or no legal therapeutic options. The extra-label use of fluoroquinolones is banned in food animals and the extra-label use of sulfonamides in lactating dairy cows is also banned. The use of gentamicin in cattle results in extended renal residues that preclude salvage slaughter of cattle in a reasonable time frame. A clear therapeutic justification and plan should be in place before exposing *Salmonella* populations to important drugs in animal and human therapy. As with *E. coli*, all *Salmonella* serotypes are intrinsically resistant to benzyl penicillins, macrolides, and lincosamides.

The susceptibility profiles reported in Tables 30.3 and 30.4 support the stance that the routine use of tetracyclines in milk replacer in calf-rearing facilities with significant *E. coli* and *Salmonella* infectious pressure is a nonsensical practice.

Table 30.5. Iowa State University 2001–2003 bovine *Salmonella* Dublin susceptibility testing results (n=35).

	Number of Isolates Displaying Each Minimal Inhibitory Concentration (µg/ml)												
Drug	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Ampicillin			1	2					32				
Apramycin						30	4	1					
Ceftiofur			20	11		1	2	1					
Chlortetracycline						4	1	30					
Clindamycin						35							
Enrofloxacin	33		1	1									
Erythromycin							35						
Florfenicol				1	5	22	3	4					
Gentamicin				35									
Neomycin						6		4	17	8			
Oxytetracycline					3	2		30					
Penicillin						1		34					
Sulfadimethoxine									1		1		33
Spectinomycin									6	1	28		
Sulfachlorpyridazine									4			14	17
Sulfathiazole									4				31
Tilmicosin								1	3	31			
Trimeth/sulfa			31		1	3							
Tylosin concentrations:					2.5	5	10	20	40				
Tylosin									35				

Shaded: concentrations tested. Dark shading: susceptibility breakpoint.

Table 30.6. Iowa State University 2001–2003 bovine *Salmonella* Newport (type C2) susceptibility testing results (n=113).

Drug	Number of Isolates Displaying Each Minimal Inhibitory Concentration (µg/ml)												
	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Ampicillin				3	1								
Apramycin						110	2	1					
Ceftiofur			5				1		107				
Chlortetracycline						2			111				
Clindamycin						113							
Enrofloxacin	111	2											
Erythromycin							113						
Florfenicol					1	1				111			
Gentamicin				54			3		56				
Neomycin						46	1					66	
Oxytetracycline					1				112				
Penicillin						1	3		109				
Sulfadimethoxine												2	111
Spectinomycin								33	18		62		
Sulfachlorpyridazine									1	1	2	30	79
Sulfathiazole										2			111
Tilmicosin							1	3	5		104		
Trimeth/sulfa			109			4							
Tylosin concentrations:					2.5	5	10	20	40				
Tylosin									113				

Shaded: concentrations tested. Dark shading: susceptibility breakpoint.

Cryptosporidium parvum

Many antimicrobials have been evaluated in *Cryptosporidium parvum* calf disease models. Antimicrobials reported as ineffective in calves up to 14 days of age when administered in milk replacer during a 10-day challenge model include amprolium, sulfadimidine, dimetridazole, metronidazole, ipronidazole, quina-crine, and monensin. Trimethoprim/sulfadiazine was also ineffective when administered daily as a bolus. Lasalocid was ineffective at 0.8 mg/kg per day. When treated with 8 mg/kg per day of lasalocid, six of ten treated calves died, with one of the four surviving calves becoming infected (Moon et al., 1982). Sulfadimethoxine has also proven ineffective against *C. parvum* in a one- to seven-day-old calf challenge model (Fayer, 1992).

Lasalocid has been used as a preventive or therapeutic agent for cryptosporidium in calves based on anecdotal reports. This use in neonatal calves has resulted in reported toxicities after 100 mg twice daily in milk replacer or 200 mg oral once-daily doses starting at birth, with death occurring after one to three administrations (Benson et al., 1998). The authors confirmed this toxicity experimentally by dosing neonatal calves once at 5 mg/kg.

In other studies, lasalocid doses of 15 mg/kg have been tolerated in calves greater than seven days old with cessation of oocyst shedding three days after the last of three daily doses of 15 mg/kg (Gobel, 1987). These data would suggest that effective doses of lasalocid are toxic in neonatal calves but may be used in calves of at least one week of age.

A trial evaluating decoquinatate in a calf *C. parvum* challenge model found a significant decrease in number of days with abnormal stool scores in the treated groups given 875 or 1750 mg (10x label dose) decoquinatate per day, but no difference in oocyst shedding or weight gain (Redman, 1994). Another challenge study found no difference in days to diarrhea, days to shedding, duration of diarrhea, or oocyst shedding in calves given 2 mg/kg decoquinatate in milk replacer (Moore et al., 2003).

In naturally occurring *C. parvum* infections, halofuginone lactate administered in the milk replacer at 60 µg/kg per day cleared all shedding of oocysts within six days after the start of treatment in 98% of the treated animals. It should be noted that 93% of the untreated controls in this study also cleared the organism within 10 days of arrival at the facility (Villacorta et al., 1991). In a natural disease model, calves receiv-

ing 5 mg of halofuginone lactate daily in milk replacer were 70% less likely to shed *C. parvum* oocysts as compared to untreated controls. Weight gain and milk and starter intakes were not significantly different between groups (Jarvie et al., 2005). In a challenge study, halofuginone reduced disease at 60 and 120 µg/kg per day but was ineffective at 30 µg/kg per day (Naciri et al., 1993).

Other antimicrobials such as paromomycin, azithromycin, and clarithromycin have been demonstrated to be efficacious in the treatment of cryptosporidiosis in murine models or human therapy (Fichtenbaum et al., 1993; Rehag, 1991; Holmberg et al., 1998). The prophylactic potential of paromomycin was also demonstrated in a calf model (Fayer and William, 1993). However, the costs of these three agents have prohibited their use in food animals.

Bibliography

- Allen LJ, George LW, Willits N. 1995. Effect of penicillin or penicillin and dexamethasone on cattle with infectious keratoconjunctivitis. *J Am Vet Med Assoc* 206:1200.
- Alt DP, Zuerner RL, Bolin CA. 2001. Evaluation of antibiotics for treatment of cattle infected with *Leptospira borgpetersenii* serovar *hardjo*. *J Am Vet Med Assoc* 219:636.
- Animal Medicinal Drug Use Clarification Act (AMDUCA). 1996. Federal Register 61:217, 57731-57746. 21 CFR Part 530, Extra-label drug use in animals; final rule, Department Of Health And Human Services, Food and Drug Administration. Docket No. 96N-0081, RIN 0910-AA47. From the Federal Register online via GPO access <http://www.gpoaccess.gov/>.
- American Veterinary Medical Association (AVMA). 2005. Judicious Therapeutic Use of Antimicrobials. <http://www.avma.org/scienact/jtua/default.asp>.
- Angelos JA, et al. 2000. Efficacy of florfenicol for treatment of naturally occurring infectious bovine keratoconjunctivitis. *J Am Vet Med Assoc* 216:62.
- Apley MD. 2003. Susceptibility testing for bovine respiratory and enteric disease. In: *The Veterinary Clinics of North America* 19:3. Food Animal Practice: Lameness. Philadelphia: WB Saunders.
- Ayars WHJ, Monke DR, and Love B. 1999. An outbreak of encephalitic listeriosis in Holstein bulls. *Bov Pract* 33:138.
- Ayling RD, et al. 2000. Comparison of in vitro activity of danofloxacin, florfenicol, oxytetracycline, spectinomycin and tilmicosin against recent field isolates of *Mycoplasma bovis*. *Vet Rec* 146:745.
- Baba E, et al. 1989. Antibiotic susceptibility of *Fusobacterium necrophorum* from bovine hepatic abscesses. *Brit Vet J* 145:195.
- Benson JE, et al. 1998. Lasalocid toxicosis in neonatal calves. *J Vet Diagn Invest* 10:210.
- Berg JN, Scanlan CM. 1982. Studies of *Fusobacterium necrophorum* from bovine hepatic abscesses: biotypes, quantitation, virulence, and antibiotic susceptibility. *Am J Vet Res* 43:1580.
- Braun U, Stehle C, Ehrensperger F. 2002. Clinical findings and treatment of listeriosis in 67 sheep and goats. *Vet Rec* 150:38.
- Campbell SG, et al. 1975. An unusual epizootic of actinobacillosis in dairy heifers. *J Am Vet Med Assoc* 166:604.
- Chenault JR, et al. 2001. Efficacy of ceftiofur hydrochloride administered parenterally for five consecutive days for treatment of acute post-partum metritis in dairy cows. 34th annual convention of the Am Assn Bov Pract, 137.
- Clinical Laboratory Standards Institute (CLSI). 2003. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: approved standard, second edition. M31-A2, ISBN 1-56238-461-9.
- Coetzee JF, et al. 2005. Comparison of three oxytetracycline regimens for the treatment of persistent *Anaplasma marginale* infections in beef cattle. *J Vet Parasit* 127:61.
- Coker PR, Smith KL, Hugh-Jones ME. 2002. Antimicrobial susceptibilities of diverse *Bacillus anthracis* isolates. *Antimicrob Agents Chemother* 46:3843.
- Constable PD. 2004. Antimicrobial use in the treatment of calf diarrhea. *J Vet Intern Med* 18:8.
- Daigneault J, George LW. 1990. Topically applied benzathine cloxacillin for treatment of experimentally induced infectious bovine keratoconjunctivitis. *Am J Vet Res* 51:376.
- Dennison AC, Van Metre DC, Callan RJ. 2002. Hemorrhagic bowel syndrome in dairy cattle: 22 cases (1997–2000). *J Am Vet Med Assoc* 221:686.
- Dowling PM, Tyler JW. 1994. Diagnosis and treatment of bacterial endocarditis in cattle. *J Am Vet Med Assoc* 204:1013.
- Dueger EL, et al. 1999. Efficacy of florfenicol in the treatment of experimentally induced infectious bovine keratoconjunctivitis. *Am J Vet Res* 60:960.
- Duran SP, et al. 1991. Comparative in-vitro susceptibility of *Bacteroides* and *Fusobacterium* isolated from footrot in sheep to 28 antimicrobial agents. *J Vet Pharmacol Therap* 14:185.
- Draxxin (tulathromycin) product label. 2005. Pfizer Animal Health.
- Fayer R. 1992. Activity of sulfadimethoxine against *Cryptosporidiosis*. *J Parasit* 78:534.
- Fayer R, William E. 1993. Paromomycin is effective as prophylaxis for cryptosporidiosis in dairy calves. *J Parasit* 79:771.
- Fichtenbaum CJ, Ritchie DJ, Powderly WG. 1993. Use of paromomycin for treatment of cryptosporidiosis in patients with AIDS. *Clin Infect Dis* 16:298.
- Food and Drug Administration Center for Veterinary Medicine. 2005. www.fda.gov/cvm. Search: prohibited extra-label use.
- Gobel E. 1987. Diagnose und therapie der akuten kryptosporidiose beim kalb (Diagnosis and treatment of acute cryptosporidiosis in the calf). *Tierärztliche Umschau* 42:863.

- Hermoso-de-Mendoza J, et al. 1994. In vitro studies of *Dermatophilus congolensis* antimicrobial susceptibility by determining minimal inhibitory and bactericidal concentrations. *Brit Vet J* 150:189.
- Hoffman L, Klinefelter T. 2000-2005. Iowa State University Diagnostic Microbiology Laboratory. Personal communications.
- Holmberg SD, et al. 1998. Possible effectiveness of clarithromycin and rifabutin for cryptosporidiosis chemoprophylaxis in HIV disease. *J Am Vet Med Assn* 279:384.
- Jang S, Hirsh DC. 1994. Characterization, distribution, and microbiological associations of *Fusobacterium* spp. in clinical specimens of animal origin. *J Clin Microbiol* 32:384.
- Jarvie BD, et al. 2005. Effect of halofuginone lactate on the occurrence of *Cryptosporidium parvum* and growth of neonatal dairy calves. *J Dairy Sci* 88:1801.
- Jousimies-Somer H, Pyorala S, Kanervo A. 1996. Susceptibilities of bovine summer mastitis bacteria to antimicrobial agents. *Antimicrob Agents Chemother* 40:157.
- Königsson K, et al. 2000. Effects of NSAIDs in the treatment of postpartum endometritis in the cow. *Reproduction in Domestic Animals* 35:186.
- Larson RL. 1997. Diagnosing and controlling seminal vesiculitis in bulls. *Vet Med Dec*:1073.
- Lechtenberg KE, Nagaraja TG, Chengappa MM. 1998. Antimicrobial susceptibility of *Fusobacterium necrophorum* isolated from bovine hepatic abscesses. *Am J Vet Res* 59:44.
- Mateos E, et al. 1997. Minimum inhibitory concentrations for selected antimicrobial agents against *Fusobacterium necrophorum* isolated from hepatic abscesses in cattle. *J Vet Pharmacol Therap* 20:21.
- McGuirk, SM. 1991. Treatment of cardiovascular disease in cattle. *Appl Pharm Therap* 7:729.
- Moon HW, Woode GN, Ahrens FA. 1982. Attempted chemoprophylaxis of cryptosporidiosis in calves. *Vet Rec* 110:181.
- Moore DA, et al. 2003. Prophylactic use of decoquinat for infections with *Cryptosporidium parvum* in experimentally challenged neonatal calves. *J Am Vet Med Assoc* 223:839.
- Mundt HC, et al. 2003. Efficacy of toltrazuril against artificial infections with *Eimeria bovis* in calves. *Parasit Res* 90 (Suppl 3):S166.
- Naciri MR, et al. 1993. The effect of halofuginone lactate on experimental *Cryptosporidium parvum* infections in calves. *Vet Parasitol* 45:199.
- Odendall MW, Pieterse PM, Vos V. 1990. The antibiotic sensitivity patterns of *Bacillus anthracis* isolated from the Kruger National Park. *J Vet Res* 58:17.
- Piriz-Duran S, et al. 1990. Susceptibilities of *Bacteroides* and *Fusobacterium* spp. from foot rot in goats to 10 beta-lactam antibiotics. *Antimicrob Agents Chemother* 34:657.
- Redman DR, Fox JE. 1994. The effect of varying levels of DECCOX on experimental cryptosporidia infections in Holstein bull calves. In: *Proceedings, 26th Ann Mtg Am Assoc Bov Pract*, 157.
- Rehg JE. 1991. Activity of azithromycin against cryptosporidia in immunosuppressed rats. *J Infect Dis* 163:1293.
- Rings DM, Rings MB. 1996. Managing cryptosporidium and giardia infections in domestic ruminants. *Vet Med Dec*:1125.
- Roby TO, Mazzola V. 1972. Elimination of the carrier state of bovine anaplasmosis with imidocarb. *Am J Vet Res* 33:1931.
- Rosenbusch RF. 1998. Antibiotic susceptibility of *Mycoplasma bovis* strains recovered from mycoplasmal pneumonia and arthritis in feedlot cattle. AS leaflet R1548, 1998 Beef Research Report. Ames, Iowa: Iowa State University.
- Rosenbusch RF, et al. 2005. Antimicrobial inhibition profiles of *Mycoplasma bovis* isolates recently recovered from various US regions and diverse clinical conditions. *J Vet Diag Invest* (in press).
- Salmon SA, Watts JL, Yancey RL Jr. 1996. In vitro activity of ceftiofur and its primary metabolite, desfuroylceftiofur, against organisms of veterinary importance. *J Vet Diag Invest* 8:332:336.
- Samitz EM, Jang SS, Hirsh DC. 1996. In vitro susceptibilities of selected obligate anaerobic bacteria obtained from bovine and equine sources to ceftiofur. *J Vet Diag Invest* 8:121.
- Sanyal, PK, Ruprah NS, and Chhabra MB. 1985. Chemotherapeutic effects of sulphadimidine, amprolium, halofuginone and chloroquine phosphate in experimental *Eimeria bareillyi* coccidiosis of buffaloes. *Vet Parasitol* 17:117.
- Sasaki Y, et al. 2001. Tetracycline-resistance genes of *Clostridium perfringens*, *Clostridium septicum* and *Clostridium sordellii* isolated from cattle affected with malignant edema. *Vet Microbiol* 83:61.
- Tripathi D, et al. 2001. Serodiagnosis and treatment of listeriosis in repeat breeder cattle. *Ind J An Sci* 71:3.
- Villacorta I, et al. 1991. Efficacy of halofuginone lactate against cryptosporidium parvum in calves. *Antimicrob Agents Chemother* 35:283.
- Zielinski GC, Piscitelli HG, Perez-Monti H. 1999. Efficacy of different dosage levels and routes of inoculation of tilmicosin in a natural outbreak of infectious bovine keratoconjunctivitis. *Proc 32nd Ann Mtg Am Assoc Bov Pract* 32:261.

Antimicrobial Drug Use in Bovine Mastitis

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The most common use of antimicrobial drugs on dairy farms is to treat mastitis (Mitchell et al., 1998). The expenses associated with mastitis (decreased milk production, decreased milk quality, drug costs, dumped milk, and difficulty resolving some mastitis infections) may be considerable, and have led many dairy producers to implement management programs focused on mastitis prevention and control. Money spent on an effective protocol for prevention of mastitis is likely to lead to an overall financial benefit to the dairy. On a farm that is experiencing high somatic cell counts, high rates of clinical mastitis, high levels of subclinical mastitis, or all of these problems, investigation into the reason for the problem followed by development and implementation of a program to alleviate the cause and prevent new occurrences is recommended as the first step in resolving the problem. Treatment alone is unlikely to solve herd-level mastitis problems.

Even on well-managed farms with mastitis prevention protocols in place, treatment of clinical mastitis may sometimes be desirable. Subclinical mastitis may be detected through a combination of individual cow Somatic Cell Counts (SCCs) as measured by DHI testing or the California Mastitis Test (CMT) and microbial culture of milk samples. Although the discussion presented here is addressed to clinical cases of mastitis, the principles described are also generally applicable to the treatment of subclinical mastitis.

Mastitis During Lactation

Cow Factors

A number of questions should be asked about the cow before deciding how or even whether to initiate treat-

ment for a case of mastitis. Depending on cow factors, one may decide to treat the mastitis using a label-prescribed or extra-label protocol, or it may be more rational not to treat the mastitis, either because treatment is unnecessary or because treatment is unlikely to result in resolution of clinical signs. Table 31.1 outlines cow factors to be considered prior to initiating antimicrobial therapy of mastitis.

Questions to ask before treatment is instituted include:

- 1) Is this a new case of mastitis or a relapse? Repeated treatment of a recurrent case of mastitis is frequently unrewarding. If the recurrent case of mastitis is to be treated, the therapeutic regimen should be more extensive than what would be used for a mild, acute case.
- 2) How severe is it? A case of mastitis in a cow that has become systemically ill (septic/toxic), will require a therapeutic protocol that includes systemic antibiotic therapy, intramammary therapy, supportive therapy and closer monitoring than a case in which clinical signs are limited to the udder and milk.
- 3) How many quarters are affected? The expense and the likelihood of treatment failure increase as the number of affected quarters increases. A cow with 3 or 4 affected quarters may have immune system deficiencies or an infection with an especially virulent or persistent bacterial strain. These complications will make the likelihood of therapeutic success small.
- 4) Stage of lactation: For a cow in late lactation, economic and therapeutic advantages may be gained by treating the cow simultaneously with drying-off.
- 5) Other health problems: It has been established that the likelihood of a cow developing mastitis is in-

Table 31.1. Factors affecting mastitis treatment decisions.

Factor	Effect
Duration	
New case	Greater likelihood of success, compared to relapse.
Relapse	Less likelihood of success, compared to new case.
Chronic infection	Low chance of therapeutic success.
Severity	
Mild case (clots/flakes)	May not require antimicrobial therapy; on-farm culture may help in decision-making.
Moderate case (mammary swelling)	May not require antimicrobial therapy, or may respond to intramammary therapy alone.
Severe case	Requires systemic antimicrobial therapy and supportive care.
Number of quarters affected	
1-2 quarters	More likely to respond to therapy or spontaneously resolve.
3-4 quarters	Less likely to respond to therapy or spontaneously resolve.
Stage of lactation	
Early lactation	Most economic benefit to successful treatment.
Mid lactation	Weigh other factors before treating: pathogen, pregnancy status, health status, production level, duration of infection.
Late lactation	May benefit from treatment at dry-off.
Health status	
Otherwise healthy	High herd value and likelihood of successful treatment.
Other health problems, lame, open	Lower value to herd; may be less likelihood of successful therapy.

creased by the presence of other health problems such as ketosis and hypocalcemia (Curtis et al., 1983). It is reasonable to expect, therefore, that the likelihood of successful therapy of mastitis is decreased in cows with concurrent illnesses.

Pathogen Factors

Microbial culture of mastitis infections is an invaluable aid in determining whether or how to institute therapy. Infections with certain pathogens are likely to respond to antibiotic therapy, while some pathogens may or may not respond to antimicrobial therapy (due to intrinsic pathogenic factors or cow factors such as those described above). Some infections are likely to resolve without any treatment, while therapy may actually delay resolution of the infection in some cases. Common mastitis pathogens which are likely to be unresponsive to antimicrobial therapy are listed in Table 31.2. A brief overview of some other commonly encountered pathogens follows.

Streptococcus agalactiae is a contagious mastitis pathogen. It is considered highly responsive to therapy with nearly any antimicrobial drug.

Chronicity decreases the responsiveness of *Staphylococcus aureus* to antimicrobial therapy. A new case in one quarter of a young cow is much more likely to respond to appropriate therapy than one or more quar-

Table 31.2. Common mastitis pathogens unlikely to respond to antimicrobial treatment.

<i>Arcanobacterium pyogenes</i>
<i>Bacillus</i>
<i>Mycobacterium</i>
<i>Mycoplasma bovis</i>
<i>Nocardia</i>
<i>Pasteurella</i>
<i>Proteus</i>
<i>Prototheca</i> (algae)
<i>Pseudomonas</i>
<i>Serratia</i>
Yeast (Antibiotic treatment will delay spontaneous cure.)

ters chronically infecting an older cow. Other *Staphylococcus* species have shown better response to therapy (Owens et al., 1997). Extra-label use extending the duration of therapy may increase the likelihood of therapeutic success for chronic or recurrent infections with *Streptococcus* or *Staphylococcus* species (Morin et al., 1998; Oliver et al., 2004).

The Gram-negative coliform organisms (*Escherichia*, *Klebsiella*, and *Enterobacter*) are variable in their clinical expression and response to antimicrobial therapy. Infections with coliform organisms may cause mild or no clinical signs and resolve on their own, but they may

also cause severe, life-threatening illness or chronic infections. As with other pathogens, treatment decisions should be based on the severity and chronicity of infection; mild acute infections may resolve with no therapy or limited therapy, whereas chronic infections may require longer term, possibly extra-label therapy, and severely ill cows will require supportive therapy in addition to treatment with antimicrobial drugs.

Mycoplasma bovis provides a unique set of circumstances as a mastitis pathogen. Mastitis caused by *Mycoplasma* may occasionally resolve without treatment, but antimicrobial therapy will not affect the outcome (Gonzalez and Wilson, 2003).

Selecting an Antimicrobial Drug

Intramammary Antimicrobial Use

After cow and pathogen factors have been weighed and the decision has been made to treat a case of mastitis, a suitable therapeutic regimen must be designed. A therapeutic regimen consists of the drug to be used, drug dose, route of administration, frequency of administration, and duration of use. For mild to moderate mastitis (abnormal milk with or without mammary swelling), antibiotic therapy is usually administered by the intramammary route, if it is administered at all. Table 31.3 lists intramammary preparations available for the treatment of lactating dairy cows in the United States.

Only eight intramammary antibiotic preparations are currently available for use in the United States. Their active ingredients are amoxicillin, ceftiofur, cephalixin, cloxacillin, erythromycin, hetacillin, penicillin, and pirlimycin.

Spectrum of activity is a key consideration when selecting an antimicrobial drug for intramammary therapy of mastitis. Intramammary use of drugs or preparations not specifically manufactured for intramammary administration is not recommended; such substances may be irritating to udder tissues and may result in inflammation and scarring. Compounded preparations are at risk for contamination with infectious pathogens, and milk and meat withholding times recommended for other routes of administration are likely to be inaccurate for intramammary administration. It is also not advised to use two different antimicrobial preparations simultaneously in one quarter, as interactions between the two drugs may decrease effi-

cacy. For example, macrolides and lincosamides bind at such close sites on the bacterial ribosome that when they are administered simultaneously, they compete for binding and the net effect of the combination of the two drugs is not additive. Consequently, simultaneous use of the macrolide drug erythromycin and the lincosamide drug pirlimycin, both of which are available in formulations for intramammary use, would not provide additional therapeutic benefit and may actually reduce efficacy as compared to either drug alone.

Erythromycin, a macrolide, and pirlimycin, a lincosamide, are the only drugs available as intramammary preparations that are not members of the beta-lactam drug class. Both macrolide and lincosamide drugs are considered to have primarily Gram-positive antimicrobial spectra, without activity against the coliform mastitis pathogens.

One of the earliest beta-lactam drugs to be developed, benzathine penicillin G, is available for intramammary administration. This drug is active against many streptococci and non-penicillinase-producing staphylococci (Vaden and Riviere, 2001). The drug is inactive against the Enterobacteriaceae (*Proteus*, *Salmonella*, *Enterobacter*, *Klebsiella*, and *Escherichia*), and resistance by *Staphylococcus* spp. is likely to be common.

Amoxicillin and hetacillin belong to the aminopenicillin group. Hetacillin is prepared by a reaction of ampicillin with acetone, and has an identical spectrum of activity to ampicillin. The aminopenicillins are active against bacteria susceptible to penicillin G, as well as some Enterobacteriaceae such as *Escherichia coli* (Vaden and Riviere, 2001). Despite being within the spectrum of activity of aminopenicillins, 30 to 50% of human *Escherichia coli* isolates are insensitive to the aminopenicillins (Petri, 2001). Resistance may also be present among some veterinary pathogens. Like the natural penicillins, aminopenicillins are susceptible to beta-lactamases produced by Gram-positive and Gram-negative bacteria (Petri, 2001). In human preparations, this susceptibility of the drugs to destruction is counteracted by the production of drug compounds containing both an aminopenicillin and a beta-lactamase inhibitor such as clavulanic acid, but such preparations are not available for intramammary administration.

Cloxacillin is a penicillinase-resistant penicillin. It is active against penicillinase-producing *Staphylo-*

coccus aureus strains that would be resistant to the natural penicillins and aminopenicillins. Against other penicillin-sensitive organisms, however, cloxacillin is less active (Petri, 2001).

Cephapirin and ceftiofur are members of the cephalosporin group of beta-lactam drugs. First-generation cephalosporin drugs such as cephapirin are generally considered to be active against *Streptococcus*, *Staphylococcus*, *Escherichia coli*, *Klebsiella*, and *Proteus*, but not against *Enterococcus* spp. (Vaden and Riviere, 2001). Third-generation cephalosporins such as ceftiofur are less active than first-generation cephalosporins against Gram-positive cocci, but more active against the Enterobacteriaceae, including those strains that produce beta-lactamase.

It must be borne in mind that even when a pathogen is considered to be within the spectrum of activity of an antimicrobial drug, the prognosis for resolution of the intramammary infection may be poor. For example, *Pasteurella* is within the spectrum of activity of several drugs available for intramammary administration, yet the prognosis for resolution of mastitis caused by *Pasteurella* is always poor (National Mastitis Council, 1999).

All of the drugs currently available as intramammary preparations work as time-dependent antimicrobials. From a pharmacodynamic standpoint, efficacy is maximized by keeping the concentration of drug at the site of infection above the level necessary to inhibit microbial growth (Minimum Inhibitory Concentration or MIC) as long as possible between doses of the drug. The drug concentration should be above the MIC for at least half the dosing interval for Gram-positive pathogens and for the entirety of the dosing interval for Gram-negative pathogens.

Once the MIC of the drug is achieved at the site of infection, increased drug concentrations above the MIC do not increase efficacy for those drugs available as intramammary preparations. Maintaining the concentration at 25% greater than MIC for a certain length of time should be as effective as maintaining the drug level at 100% above the MIC for the same time period. Consequently, if one wishes to prescribe extra-label therapy for a case of mastitis that may be difficult to resolve using label dosing regimens, extending the duration of therapy (provided drug concentrations are maintained above the MIC between doses) is expected to be more effective than giving a higher dose at each treatment time without extending

the duration of therapy. The only exception to this rule would be if the drug was cleared so slowly that drug accumulation following a higher dose resulted in the drug concentration remaining above the MIC for additional dosing intervals. Available mastitis preparations are unlikely to accumulate in the mammary gland to the point that administering 2 tubes will result in extension of therapeutic concentrations for one or more additional dosing intervals.

Regardless of the approach, it is critical that extra-label use of any drug in a food animal such as a dairy cow be accompanied by extended milk and meat withholding times. For help in setting extended withholding times following extra-label use, the Food Animal Residue Avoidance Databank provides free assistance. They can be reached in the United States by dialing 1-888-US-FARAD.

Systemic Antimicrobial Use

For acute mild to moderate mastitis, systemic antimicrobial therapy is not generally indicated or undertaken. For severe cases of mastitis (those that involve systemic clinical signs such as fever or depression in addition to abnormal milk and udder swelling), systemic administration of antimicrobial drugs is an appropriate part of therapy. Supportive care by administration of fluids and other methods is also critical in such cases, and has been discussed elsewhere (Morin, 2004). Mastitis with systemic illness is commonly caused by coliform organisms such as *Escherichia coli* and *Klebsiella*. An investigation of naturally occurring cases of coliform mastitis with systemic illness has demonstrated that 42% of cows with severe illness due to coliform mastitis had concurrent bacteremia (Wenz et al., 2001). Although systemic illness due to mastitis is frequently caused by Gram-negative pathogens, it may also be caused by Gram-positive pathogens such as *Staphylococcus aureus* (Erskine et al., 2002). Because microbial culture generally takes 24 hours to yield a preliminary result, therapy of severe mastitis must initially be based on the possibility of a Gram-positive or Gram-negative bacterial etiology. For mastitis due to coliform infection, research suggests that by the time clinical signs appear, bacterial numbers in the mammary gland have already peaked (Erskine et al., 1989). Consequently, a rational approach to therapy of severe acute mastitis would be to address the possibility of coliform bacteremia by using a systemic drug with a good spectrum of activity against Gram-negative

Table 31.3. Intramammary preparations available for lactating cows in the United States.

Drug Name and Class	Product Name	Label Regimen and Indications	Other Label Claims
Amoxicillin Aminopenicillin	Amoxi-Mast® (Schering Plough Animal Health)	3 treatments at 12-hour intervals. Subclinical <i>Staphylococcus aureus</i> mastitis. Subclinical <i>Streptococcus agalactiae</i> mastitis.	Susceptibility shown by <i>Escherichia coli</i> in vitro. Most <i>Pseudomonas</i> , <i>Klebsiella</i> , and <i>Enterobacter</i> are resistant.
Ceftiofur 3rd-generation cephalosporin	Spectramast® (Pfizer Animal Health)	2 to 8 treatments at 24-hour intervals. Clinical coagulase-negative <i>Staphylococcus</i> mastitis. Clinical <i>Streptococcus dysgalactiae</i> mastitis. Clinical <i>Escherichia coli</i> mastitis.	
Cephapirin 1st-generation cephalosporin	Today®, Cefa-lak® (Fort Dodge Animal Health)	2 treatments at a 12 hour-interval. Mastitis in lactating cows.	Shown to be efficacious against susceptible strains of <i>Streptococcus agalactiae</i> and <i>Staphylococcus aureus</i> .
Cloxacillin Penicillin (penicillinase-resistant)	Dariclox® (Schering Plough Animal Health)	3 treatments at 12 hour-intervals. Clinical <i>Staphylococcus aureus</i> mastitis (non-penicillinase-producing strains). Clinical <i>Streptococcus agalactiae</i> mastitis.	There is laboratory evidence that cloxacillin is resistant to destruction by penicillinase-producing organisms.
Erythromycin Macrolide	Gallimycin®-36 (Agri-Labs) Gallimycin®-36 (Durvet)	3 treatments at 12 hour intervals. Clinical <i>Staphylococcus aureus</i> mastitis. Clinical <i>Streptococcus agalactiae</i> mastitis. Clinical <i>Streptococcus dysgalactiae</i> mastitis. Clinical <i>Streptococcus uberis</i> mastitis.	Works against both acute and chronic cases.
Hetacillin Aminopenicillin	Hetacin®-K Intramammary Infusion (Fort Dodge Animal Health)	3 treatments at 24 hour-intervals; acute, chronic or subclinical mastitis.	Shown to be efficacious in treatment of mastitis in lactating cows caused by susceptible strains of <i>Streptococcus agalactiae</i> , <i>Streptococcus dysgalactiae</i> , <i>Staphylococcus aureus</i> , and <i>Escherichia coli</i> .
Penicillin	Masti-Clear® (G.C. Hanford)	Not more than 3 treatments at 12-hour intervals. Clinical <i>Streptococcus agalactiae</i> mastitis. Clinical <i>Streptococcus dysgalactiae</i> mastitis. Clinical <i>Streptococcus uberis</i> mastitis.	
Pirlimycin Lincosamide	Pirsue® Aqueous Gel (Pfizer Animal Health)	2 treatments at a 24 hour interval for clinical and subclinical mastitis.	Has been proven effective only against <i>Staphylococcus</i> species such as <i>S. aureus</i> and <i>Streptococcus</i> species such as <i>S. dysgalactiae</i> and <i>S. uberis</i> .

Note: Although every effort has been made to ensure that the information presented here is accurate and complete, the authors cannot bear responsibility for any errors or omissions. Readers are advised to contact drug manufacturers and/or read package inserts for complete information about the products listed.

pathogens, combined with an intramammary preparation that will have activity against Gram-positive pathogens.

Any systemic use of an antimicrobial drug as a therapy for mastitis will be an extra-label use, as there is currently no antimicrobial drug labeled for systemic administration for mastitis in the United States. Extra-label drug use in food animals requires extending meat and milk withholding periods. Drugs available for use in lactating dairy cattle, with appropriate spectra of

activity against coliform bacteria, include oxytetracycline, sulfadimethoxine, ceftiofur, ampicillin, and amoxicillin.

Although tetracyclines have both Gram-positive and Gram-negative activity in their spectrum of activity, some coliforms and *Staphylococcus* spp. may not be susceptible (Riviere and Spoo, 2001). Similarly, drugs such as sulfadimethoxine, the only sulfonamide drug approved for use in dairy cows, have a spectrum of activity that encompasses both Gram-negative and

Gram-positive pathogens, yet many previously susceptible organisms have developed resistance over the many years sulfa drugs have been in use (Spoo and Riviere, 2001). In addition, the use of sulfadimethoxine to treat mastitis in a lactating cow is illegal in the United States, as mastitis is not a labeled indication for the drug and extra-label use of sulfonamides in lactating dairy cows is prohibited by the regulations codified in the Animal Medicinal Drug Use Clarification Act.

The aminopenicillins, ampicillin and amoxicillin, have a spectrum of activity that includes the Enterobacteriaceae. They would therefore seem to be an appropriate choice for systemic antimicrobial therapy of cows with acute severe mastitis. Unfortunately, therapeutic failure may be encountered due to the production of beta-lactamases by *Staphylococcus* spp., *Escherichia coli*, and *Klebsiella*.

Ceftiofur, a third-generation cephalosporin, also has a spectrum of activity that includes coliform mastitis pathogens, and it is relatively resistant to beta-lactamases produced by those bacteria. When used in combination with intramammary antimicrobial drugs, anti-inflammatory drugs, and other supportive therapy, the addition of intramuscular ceftiofur to the treatment regimen for severe acute mastitis decreased the likelihood of a cow subsequently dying or being culled (Erskine et al., 2002).

Questions frequently arise about florfenicol and the macrolide drug tilmicosin for systemic use against coliform mastitis, as they are active against the Gram-negative pathogens that cause respiratory disease in cattle. These drugs are not good choices for Gram-negative septicemia associated with severe mastitis, however. Although the Gram-negative respiratory pathogens, *Mannheimia* and *Pasteurella*, may be susceptible to these drugs, the Gram-negative coliform organisms that commonly cause mastitis are either entirely unsusceptible to them, or the MIC is so high that administration of an impossibly high dose would be necessary to obtain any benefit.

Susceptibility Testing and Mastitis

Susceptibility testing is a way of quantifying the interaction between microbes and antimicrobial drugs in the laboratory. Susceptibility testing may be performed using serial dilution or agar gel diffusion (Kirby-Bauer) methods. For the serial dilution method, broth cultures

of the organism under examination are exposed to various concentrations of the antimicrobial drug or drugs under test. The lowest concentration of the drug(s) that inhibits microbial growth, if there is a concentration that does so, is called the Minimum Inhibitory Concentration (MIC). Note that this may not be the same as the concentration of drug necessary to sterilize the culture, which is called the Minimum Bactericidal Concentration (MBC). Using the MIC instead of the MBC to draw conclusions about antimicrobial efficacy is consistent with the therapeutic goal: to assist the cow's own immune system in clearing the infection.

Breakpoints are used to classify MICs as indicators of microbial susceptibility or resistance. For some combinations of drug and microbe, MIC results may be reported as susceptible, intermediate, or resistant. For example, when testing the susceptibility of *Streptococcus* spp. other than *Streptococcus pneumoniae* against the drug erythromycin, isolates that are inhibited at a drug concentration of 0.25 µg/ml are categorized as susceptible, while isolates that require drug concentrations of 1 µg/ml or more are classified as resistant. Isolates that have an MIC of 0.5 µg/ml would therefore be reported as intermediate.

The Kirby-Bauer method of susceptibility testing is based on placing an antibiotic disk containing a known amount of the drug under test onto an agar gel plate inoculated with the organism under test. The area around the disk where microbial growth is inhibited, if there is such an area, is called the zone of inhibition, and the diameter of this zone is used as the breakpoint to classify microbes as susceptible, intermediate, or resistant to the drugs tested. The breakpoints for the Kirby-Bauer method are extrapolated from the breakpoints used in susceptibility testing by serial dilution. Proper execution of either method of susceptibility testing requires skill, training, attention to detail, and quality control measures. For accurate results, it is recommended that samples for susceptibility testing be submitted to a veterinary diagnostic laboratory accredited by the American Association of Veterinary Laboratory Diagnosticians.

Even when properly executed, susceptibility testing has limited value as an aid to therapeutic decision-making in bovine mastitis. The relationship between susceptibility as determined by laboratory susceptibility testing and the outcome of clinical cases of mastitis appears to be inconsistent at best. Many publications have described patterns of susceptibility seen in the

laboratory, but few of these publications assess whether susceptibility in the laboratory has predictive value for clinical outcomes of mastitis (Constable and Morin, 2003). The issue is further complicated by the use of variable outcomes in trials assessing resolution of clinical mastitis. For mastitis, a more practical approach to assessing therapeutic success is to design farm protocols for treatment of clinical mastitis, then periodically evaluate the protocols for efficacy.

Herd-Based Therapeutic Protocols

Modern management strategies frequently involve a standardized approach to mastitis prevention and therapy. Key benefits to standardizing the farm approach to mastitis therapy are that treatment decisions are made in advance instead of "cow-side" and that a consistent approach to therapy on the farm is developed. This results in simpler, less time consuming tasks on the farm, from deciding whether or not to treat a case of mastitis to selecting a drug and regimen and assigning an appropriate withholding time for milk and meat from treated cows. Moreover, when treatments are standardized and good records are kept, evaluating whether or not a given treatment is successful on the farm is greatly simplified.

Microbial culture of clinical mastitis cases can be a boon to designing a farm protocol for treatment. By culturing all new cases of mastitis, the organisms that are causing clinical mastitis on the farm can be identified and an appropriate therapeutic regimen designed for treatment. This is in addition to the benefit that microbial culture has in directing efforts at prevention to appropriate areas. Even if microbiological culture is not performed on every case of mastitis on a particular farm, periodic cultures are still useful as a guide to the development of treatment strategies and protocols. Culture of chronic mastitis cases is also an irreplaceable aid to determining whether a chronic case might be resolved by antimicrobial therapy, or if the pathogen causing the mastitis is not amenable to therapy and the greatest financial benefit to the farm would be not to treat.

Although some level of microbial culture is recommended in order to inform decision-making on all farms facing clinical mastitis, some farms have incorporated routine culture of every case of mastitis into their treatment protocol. Use of plates with two types of agar

gel, blood agar and MacConkey's agar, affords farms a simple classification system for all cases of mastitis following a 24-hour milk culture. Each case of mastitis may be classified as no-growth, Gram-positive, Gram-negative, or contaminated, and treatment regimens may be designed for each classification. This approach does require effort and organization, but the financial rewards to a farm may be significant. No-growth results are typically obtained for 25% to 50% of all cases of clinical mastitis, and antimicrobial therapy is probably not indicated in such cases. Decreasing the number of treated cows by up to half on farms that have previously treated every case of mastitis may result in substantial financial benefit to the farm, even after the cost of conducting microbial culture of each new case is factored in. Some farms have also decided not to treat cows with Gram-negative pathogens on culture; this practice may reduce the percentage of clinical mastitis treated to 30% or less of all new cases, depending on the predominant pathogens on the farm. If this approach is taken, it is imperative that it be undertaken in the knowledge that Gram-negative mastitis, although it will frequently resolve on its own, may also develop into a chronic infection or severe illness. Whichever culture-based treatment protocol is adopted, it is prudent to save pre-treatment milk samples in the freezer for submission to a diagnostic laboratory for definitive organism identification, in the event that the case of mastitis does not resolve.

An example of a herd protocol for mastitis is given in Figure 31.1.

Antimicrobial Therapy of Dry Cows

The dry (non-lactating) period of the lactation cycle is a critical time for dairy cattle. The major proportion of calf growth occurs during this time. Balanced nutrition, especially for cows two to three weeks before calving, is essential for prevention of post-parturient metabolic disease. The udder also undergoes marked biochemical, cellular and immunological changes during the dry period. Involution of the mammary parenchyma begins 1 to 2 days after the end of lactation and continues for 10 to 14 days. During this time, the gland is particularly vulnerable to new intramammary infections (IMI). The periparturient period and the early dry period constitute the times of greatest risk for new IMI in the lactation cycle of the cow. Once

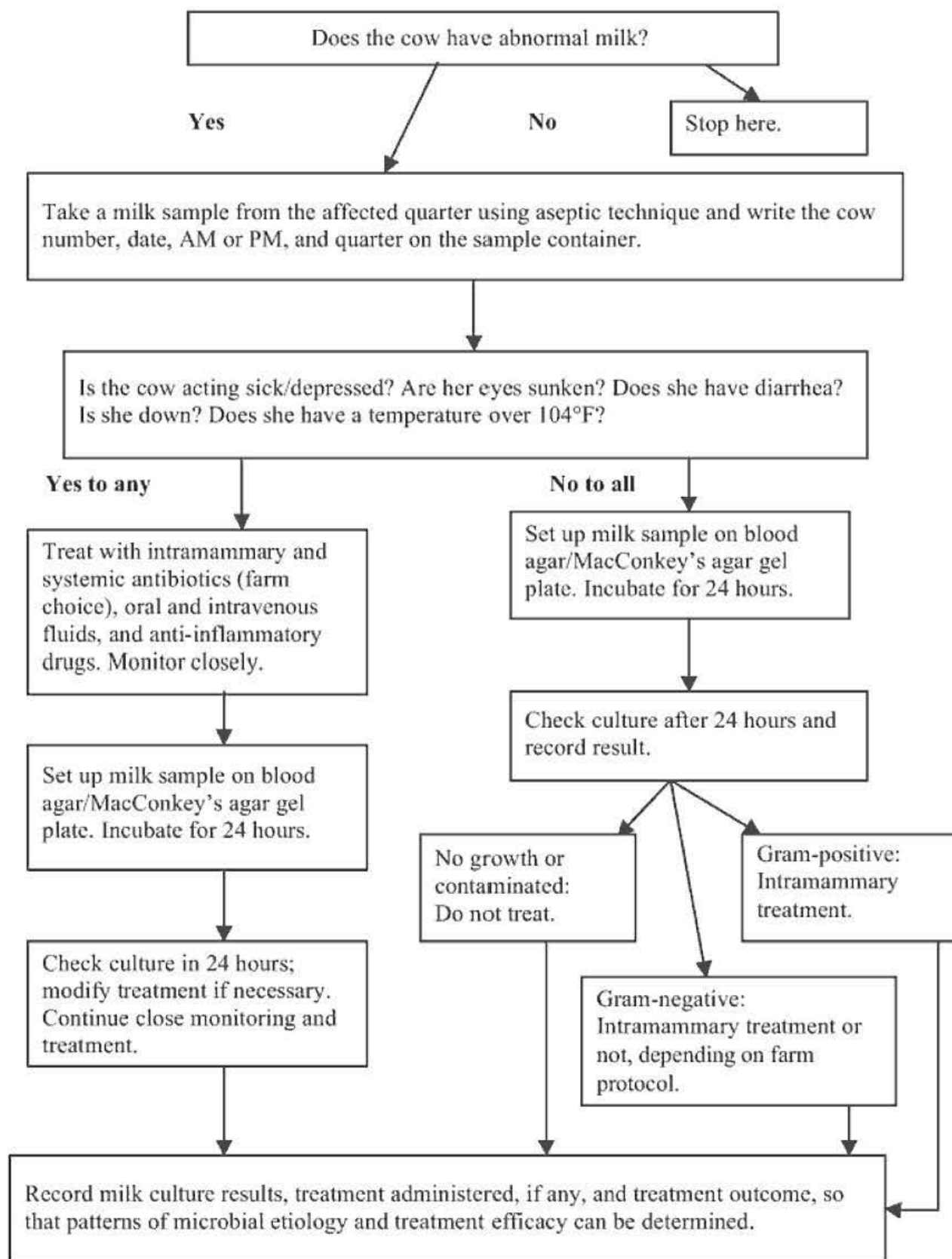


Figure 31.1. Herd mastitis treatment template.

involution is complete, however, a more hostile immune environment for bacterial pathogens exists. The most important defense, as in lactating cows, remains the teat canal. This barrier is enhanced during the dry period by the formation of a keratin plug. Additionally, there is an increase in the numbers of macrophages and lymphocytes, and concentrations of complement and immunoglobulins that can help orchestrate more efficient phagocytosis. Lactoferrin, a potent iron chelating protein, also markedly increases in dry cow secretions, thus helping to inhibit growth of Gram-negative bacteria, particularly *E. coli*. Consequently, the dry period is an ideal time to attain synergy between antibacterial therapy and immune function to eliminate pathogens from the gland, without incurring the extensive milk withholding costs typical of lactating cow therapy.

Intramammary administration of antibacterial drugs at the end of lactation has been a standard of dairy mastitis management for 35 years. Cure rates for IMI caused by all Gram-positive cocci (those IMI that existed prior to the dry period, but were not detected following calving) have been reported in numerous studies to average 75%. However, the efficacy of conventional dry cow treatments in eliminating chronic IMI is realistically closer to 15 to 30%. Most commercial dry cow products have little or no activity against Gram-negative pathogens, thus cure rates for coliform organisms are low. In one study, cows treated with a product with significant activity against Gram-negative bacteria had decreased clinical coliform mastitis during the dry period and early lactation as compared to cows treated with cloxacillin (Bradley and Green, 2001).

Because of concern regarding overuse of antibacterial drugs and potential effects on antimicrobial resistance of bacteria, selective dry cow therapy (treatment of infected cows only) versus total or blanket dry cow therapy (treatment of all cows) has been discussed. Decisions should be made on an individual herd basis, and results monitored to determine the success of a dry cow mastitis program based on numbers of new IMI during the dry period, cures of existing infections, and impact on the rate of clinical mastitis, particularly in early lactation. An important role of dry cow therapy, in addition to eliminating existing IMI, is the prevention of new IMI. Intramammary infusion of tilmicosin reduced new infection rates by greater than 33% in a Canadian study (Dingwell et al., 2002). Selective

dry cow therapy can result in more clinical mastitis in the dry period, more new IMI during the dry period, and subsequently more clinical mastitis in early lactation as compared to herds treated with blanket dry cow therapy (Berry and Hillerton, 2002). Additionally, a recent review of the literature determined that, to date, no evidence exists that supports the concept of emerging antimicrobial resistance in mastitis pathogens. Thus, the evidence suggests that for most herds, total dry cow therapy is preferred over selective dry cow therapy.

As with therapy during lactation, the use of systemic administration as an adjunct to intramammary infusion has stimulated interest in potential alternative therapeutic regimens. Subcutaneous norfloxacin nicotinate administered at the start of the dry period achieved a better cure rate and lower new infection rate over the dry period for *S. aureus* infections, as compared to untreated cows and cows administered intramammary cephapirin benzathine preparations (Soback et al., 1990; Sol et al., 1990). However, the systemic administration of tilmicosin resulted in lower drug concentrations in milk and lower cure rates for *S. aureus* mastitis than intramammary administration (Nickerson et al., 1999). Additionally, in a Michigan study, cows administered intramuscular oxytetracycline and intramammary cephapirin did not have better cure rates for quarters infected with *S. aureus* than cows treated with cephapirin only (Erskine et al., 1994). Clinical failure in these trials reflects the importance of designing a therapeutic regimen that will maintain an effective concentration of the drug at the site of infection for an adequate duration, and the poor prognosis of chronic infections. Systemic therapy should be approached judiciously, using sound pharmacological principles.

Enhancement of immune function and clearance using immune modulators administered alone or with antimicrobial drugs is also an area of potential future importance for mastitis therapy. Numerous cytokines, which regulate cell function during immune and inflammatory processes, have recently been identified and purified from cattle. With subsequent identification of the genes responsible for cytokine synthesis, recombinant production has been made possible. Cytokines that have demonstrated promise for use in the prevention and/or treatment of bovine mastitis are interleukin-1 (IL-1) and interleukin-2 (IL-2), interferons, and colony stimulating factor (Sordillo et al.,

1991). However, field trials using intramammary IL-2 as an adjunct therapy with intramammary cephalixin determined little or no benefit of IL-2 in curing or preventing IMI in the dry period; further, IL-2 has been associated with abortions in treated cattle 3 to 5 days after infusion (Erskine et al., 1998). Future research may elucidate potential uses of immune modulation but, because this class of bioagents is potent and has complex actions, care will be necessary to attain the desired antibacterial effect without adverse side effects.

In summary, important considerations for dry cow treatment include these: (1) Commercial dry cow treatments are generally effective against Gram-positive cocci in preventing, as well as eliminating IMI. (2) Due to enhanced immune function and decreased discarded milk costs, dry cows should be preferentially treated over lactating cows for subclinical and chronic IMI. (3) Most commercial intramammary products have little efficacy against Gram-negative pathogens. And (4) treatment of more chronic IMI may include systemic drug regimens, preferably with antimicrobials that distribute well in mammary tissue, such as tetracyclines and macrolides.

Antimicrobial Therapy of Heifers

By convention, heifers are considered essentially free of IMI before calving, and except for the rare case, they need little therapeutic attention until after the onset of lactation, when the risk of infection is increased. The majority of IMI in calving heifers are caused by staphylococcal species other than *S. aureus*, which have a high rate of spontaneous cure.

However, it is apparent that under some herd conditions, a substantial portion of heifers at calving are infected; additionally, some of the IMI can be caused by pathogens such as *S. aureus*. There is a geographic element of risk to this problem: Fly bite dermatitis of the teat end, which is an important physical barrier to infection, may play a role in the pathogenesis. The most significant potential problems have been reported in relation to IMI caused by *Staphylococcus* spp., which are known to colonize skin and readily increase in numbers in the presence of lesions. Clinical investigations have indicated that intramammary infusions of antibiotics 7 days before expected calving dates will reduce the rate of IMI at calving (Oliver et al., 1992; Nickerson et al., 1995). As with cows, strict

teat end antisepsis should be followed before intramammary infusion to prevent any contamination. Additionally, labor to handle animals for treatment can be extensive, and there is an increased risk of antibacterial residues in milk following calving as compared to untreated heifers. This is not a recommended management program for many dairies. However, if herd records indicate that an undesirable proportion of first-lactation animals are infected at calving, particularly with *Staphylococcus* spp., this regimen may be suitable to reduce economic losses.

Bibliography

- Berry EA, Hillerton JE. 2002. The effect of selective dry cow treatment on new intramammary infections. *J Dairy Sci* 85: 2512.
- Bradley AJ, Green MJ. 2001. An investigation of the impact of intramammary antibiotic dry cow therapy on clinical coliform mastitis. *J Dairy Sci* 84:1632.
- Constable PD, Morin DE. 2003. Treatment of clinical mastitis: using antimicrobial susceptibility profiles for treatment decisions. *Vet Clin Food Anim* 19:139.
- Curtis CR, et al. 1983. Association of parturient hypocalcemia with eight periparturient disorders in Holstein cows. *J Am Vet Med Assoc* 183:559.
- Dingwell RT, et al. 2002. The efficacy of intramammary tilmicosin at drying-off, and other risk factors for the prevention of new intramammary infections during the dry period. *J Dairy Sci* 85:3250.
- Erskine RJ, et al. 1989. Induction of *Escherichia coli* mastitis in cows fed selenium-deficient or selenium-supplemented diets. *Am J Vet Res* 50:2093.
- Erskine RJ, et al. 1994. Efficacy of intramuscular oxytetracycline as a dry cow treatment for *Staphylococcus aureus* mastitis. *J Dairy Sci* 77:3347.
- Erskine RJ, et al. 1998. Recombinant bovine interleukin-2 and dry cow therapy: Efficacy to cure and prevent intramammary infections, safety, and effect on gestation. *J Dairy Sci* 81:107.
- Erskine RJ, et al. 2002. Efficacy of systemic ceftiofur as a therapy for severe clinical mastitis in dairy cattle. *J Dairy Sci* 85:2571.
- Gonzalez RN, Wilson DJ. 2003. Mycoplasmal mastitis in dairy herds. *Vet Clin Food Anim* 19:199.
- Mitchell JM, et al. 1998. Antimicrobial drug residues in milk and meat: causes, concerns, prevalence, regulations, tests, and test performance. *J Food Prot* 61:742.
- Morin DE, et al. 1998. Comparison of antibiotic administration in conjunction with supportive measures versus supportive measures alone for treatment of dairy cows with clinical mastitis. *J Am Vet Med Assoc* 213: 676.
- Morin DE. 2004. Beyond antibiotics—what else can we do? *Proc 43rd Ann Mtg National Mastitis Council*. Charlotte, North Carolina.

- National Mastitis Council. 1999. Laboratory Handbook on Bovine Mastitis. Madison, Wisconsin: National Mastitis Council, Inc.
- Nickerson SC, et al. 1995. Mastitis in dairy heifers: initial studies on prevalence and control. *J Dairy Sci* 78:1607.
- Nickerson, SC, et al. 1999. Comparison of tilmicosin and cephalixin as therapeutics for *Staphylococcus aureus* mastitis at dry-off. *J Dairy Sci* 82:696.
- Oliver SP, et al. 1992. Influence of prepartum antibiotic therapy on intramammary infections in primigravid heifers during early lactation. *J Dairy Sci* 75:406.
- Oliver SP, et al. 2004. Extended ceftiofur therapy for treatment of experimentally-induced *Streptococcus uberis* mastitis in lactating dairy cattle. *J Dairy Sci* 87:3322.
- Owens WE, et al. 1997. Comparison of success of antibiotic therapy during lactation and results of antimicrobial susceptibility tests for bovine mastitis. *J Dairy Sci* 80:313.
- Petri WA Jr. 2001. Penicillins, cephalosporins, and other β -lactam antibiotics. In: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 10th ed., 1189-1218. Brunton L, et al., eds. New York: McGraw-Hill.
- Riviere JE, Spoo JW. 2001. Tetracycline antibiotics. In: Veterinary Pharmacology and Therapeutics, 8th ed., 828-840. Adams HR, ed. Ames, IA: Blackwell Publishing.
- Sobach S, et al. 1990. Systemic dry cow therapy in control of subclinical *Staphylococcus aureus* infections. *Proc Int Symp Bovine Mastitis*, p134.
- Sol J, et al. 1990. Factors affecting the result of dry cow treatment. *Proc Int Symp Bovine Mastitis*, p118.
- Sordillo LM, et al. 1991. Potential role of cytokines in determining the outcome of acute coliform mastitis. *Proceedings 30th Annl Meeting Natl Mastitis Council*, Arlington, VA, p51.
- Spoo JW, Riviere JE. 2001. Sulfonamides. In: Veterinary Pharmacology and Therapeutics, 8th ed., 796-817. Adams HR, ed. Ames, IA: Blackwell Publishing.
- Wenz JR, et al. 2001. Bacteremia associated with naturally occurring acute coliform mastitis in dairy cows. *J Am Vet Med Assoc* 219:976.
- Vaden SE, Riviere JE. 2001. Penicillins and related β -lactam antibiotics. In: Veterinary Pharmacology and Therapeutics, 8th ed., 818-827. Adams HR, ed. Ames, IA: Blackwell Publishing.

Antimicrobial Drug Use in Sheep and Goats

Christine B. Navarre and Shonda Marley

The principles of prudent antimicrobial selection in sheep and goats are the same as other species. Unfortunately, the list of approved antimicrobial agents for sheep and goats is short, and clinical trial data are practically nonexistent. All sheep and goats, even if considered pets by the owners, are considered food animals by the United States Food and Drug Administration's Center for Veterinary Medicine (FDA-CVM). So, when veterinarians prescribe antimicrobial agents based on therapeutic goals in sheep and goats, they must keep in mind the potential for causing residues in meat and milk. This chapter contains information to help the veterinarian select appropriate antimicrobials for different conditions in sheep and goats, while avoiding violative residues in food.

General Recommendations

Antimicrobial selection based on bacterial culture and antimicrobial susceptibility testing is always desirable, but sheep and goats are very easily stressed. The veterinarian should weigh the benefits of obtaining a sample (for example, a transtracheal wash for pneumonia) with the detrimental effects of stress caused by the sampling, which can sometimes lead to the death of the animal. Even if culture samples are obtained, results usually take at least two days to acquire, so empirical therapy is needed initially. This should be based on knowledge of the most common pathogens isolated from the diseased area, the expected antimicrobial susceptibility of those organisms, and the behavior of the antimicrobial(s) in the animal species and target tissue. Tables 32.1 and 32.2 contain information to help make these decisions.

Once an antimicrobial drug is selected, proper ad-

ministration is important. The label dose, route of administration, frequency and duration of therapy should be followed in most cases, even if labeled only for another food animal species (Table 32.3). It is also important to administer parenteral antimicrobial drugs in a way that minimizes damage to meat. Volume of drug per injection site should be limited to five milliliters. The subcutaneous, oral or intravenous routes should be selected over the intramuscular route if possible. Intramuscular injections should be given only in the neck. Subcutaneous injections should be given in the neck also. Small volumes (< 5 ml) can be given subcutaneously in the axilla or the medial aspect of the thigh.

Since follow-up treatment will often be given by owners, they should be counseled on proper drug handling and administration, and the potential for disease transmission and injection site abscesses if needles are reused. Tilimicosin, which is approved for use in sheep, can cause fatal reactions in humans and goats. This drug should not be dispensed without careful consideration of the safety issues. The manufacturer of tilimicosin provides education materials for owners to read and sign, indicating that they understand the potential for toxicity.

Administration of antimicrobials orally via food and water for *treatment* of infections should be avoided. Intake is hard to control, especially in ill animals in which intake may be decreased. Use of antimicrobials in feed and water is indicated for *prevention* of some conditions, such as coccidiosis and infectious abortion.

Residue Avoidance and Extra-label Drug Use

According to the FDA-CVM's Green Book, which lists all approved uses of veterinary drugs, there are only

Table 32.1. In vitro antimicrobial susceptibility of aerobic bacterial isolates from sheep and goats.

Organisms	# of isolates tested	Percent susceptible isolates*																
		AMI	AMC	AMP	CEP	CHL	CHT	ERY	GEN	OXA	OXY	PEN	SUL	SPYR	SDIM	STHI	TET	TMS
Gram-Negative																		
<i>Escherichia coli</i>	149	94	100	65	62	100	39	0	85		28		69	44	32	23	43	86
<i>Mannheimia haemolytica</i>	177	94	100		100	100	95	4	95		80		94	48	50	14	90	97
<i>Pasteurella multocida</i>	69	97			100		97	7	97		87		50	20	24	16	97	96
<i>Pasteurella</i> spp.	24						80	8	100		75		75	50	47	0	100	100
<i>Pasteurella trehalosi</i>	28	100			50		54	0	100		31			77	70	38	50	100
<i>Pseudomonas aeruginosa</i>	15				0		67	0	100		33		22	0	0	33	8	9
<i>Salmonella</i> spp.	83	95	73	84	90	91	71	0	100		83		64	36	28	54	78	100
Gram-Positive																		
<i>Arcanobacterium pyogenes</i>	17				100			94	100				50	0	0		71	65
<i>Corynebacterium pseudotuberculosis</i>	36	25			100		89	100	91		89		100	57	53	89	100	100
<i>Enterococcus</i>	11		100	91		50	50	38	36		50	73		0	0	0	100	
<i>Listeria monocytogenes</i>	4						100	100	100		75			50	50	50		100
<i>Staphylococcus aureus</i>	49	100		35	86		80	86	98	50	80	33	83	38	29	0	86	94
<i>Staphylococcus</i> spp.	8			63			100	75	86		86	57	100	29	29	0	100	75
<i>Coagulase negative staphylococcus</i>	22			40	71	100		60	100	64		17	63				77	80
<i>Streptococcus</i> spp.	30			87	67		44	83	88		36	77	0	25	27	0	60	68

*AMI, amikacin; AMC, amoxicillin-clavulanic acid; AMP, ampicillin; CEP, cephalothin; CHL, chloramphenicol; CHT, chlortetracycline; ERY, erythromycin; GEN, gentamicin; OXA, oxacillin; OXY, oxytetracycline; PEN, penicillin; SUL, sulfonamides; SPYR, sulfachloropyridazine; SDIM, sulfadimethoxine; STHI, sulfathiazole; TET, tetracycline; TMS, trimethoprim-sulfamethoxazole.

Sources: University of Minnesota Veterinary Diagnostic Laboratory, Colorado State University Diagnostic Laboratory, Auburn University Bacteriology Laboratory, and Iowa State University Diagnostic Laboratory (1993-2004).

Table 32.2. Antimicrobial drug selection for common conditions of sheep and goats.

Condition	Species	Infecting Organism	Suggested Antimicrobial(s)	Comments
Infectious abortion				
Enzootic abortion of ewes (EAE)	Sheep and goats	<i>Chlamydophila psittaci</i>	Tetracycline	<p><i>Prophylaxis in high risk flocks:</i> tetracycline in feed for 2 to 3 weeks prior to breeding at a dose of 200-400 mg/head/day until lambing.</p> <p><i>Outbreak:</i> 400-500 mg/head/day tetracycline in feed until lambing finished. Poor efficacy if placental damage already present.</p> <p>Not recommended for dairy goats because of milk withdrawal. Vaccination should also be considered.</p> <p><i>Outbreak or previous diagnosis:</i> long acting oxytetracycline at label dosage starting 1-3 wks before start of lambing every 10 to 14 days until finished.</p> <p><i>Outbreak:</i> 20 mg/kg IM once or twice per day.</p>
Campylobacter abortion (Vibriotic)	Sheep	<i>C. fetus</i> spp. <i>fetus</i> , <i>C. jejuni</i>	Oxytetracycline Tylosin	<p><i>Prophylaxis:</i> injections of penicillin-streptomycin for 2-5 days or tetracycline in feed as in EAE. Antimicrobial sensitivity patterns should be established from any isolates.</p> <p>Vaccination in the face of an outbreak also very successful.</p> <p><i>Outbreak:</i> long-acting formulation at 20 mg/kg every 48 hours.</p> <p><i>Outbreak:</i> 30 mg/kg IM once a day.</p> <p><i>Outbreak:</i> 100 mg/kg PO once a day.</p>
			Penicillin G-streptomycin; tetracycline	
Listeria abortion	Sheep and goats	<i>L. monocytogenes</i>	Oxytetracycline Tylosin	<p>Injectable long-acting tetracycline to all animals at risk in the face of an outbreak.</p> <p>Mixed in feed at a dose of 15 mg/head/day from breeding to lambing.</p> <p>Mixed in feed or premix to feed at a dose of 2 mg/kg/day from breeding to lambing.</p> <p>Should also prevent contamination of feed by cats and kittens.</p> <p>Vaccine available in some countries.</p>
			Sulfamethazine Oxytetracycline	
Toxoplasma abortion	Sheep and goats	<i>T. gondii</i>	Monensin	<p>Often widespread by the time diagnosis is made.</p> <p>Antibiotics may not eliminate organism; consider culling and environmental management.</p>
Salmonella abortion	Sheep and goats	<i>S. typhimurium</i> , <i>S. abortus ovis</i> , <i>S. montevideo</i> , <i>S. dublin</i>	Decoquinolate	<p>Treat all pregnant animals at risk with injections.</p>
			IM or SC broad-spectrum antimicrobials ^a	
Leptospira abortion	Sheep and goats	<i>L. hardjo</i> , <i>L. pomona</i>	Penicillin G-streptomycin; tetracyclines	<p>Abortions are more common in goats than in sheep.</p> <p>Long-acting injectable (IM or SC) to all pregnant does every 10 to 14 days until kidded.</p> <p>Watch withdrawal for milk in dairy goats.</p>
Coxiellosis (Q fever)	Sheep and goats	<i>C. burnetii</i>	Tetracycline; fluoroquinolone	
Other infectious reproductive disorders				
Metritis	Sheep and goats	<i>A. pyogenes</i> , <i>E. coli</i> , mixed anaerobes including <i>Clostridium</i> spp.	Penicillin G; ceftiofur; broad-spectrum antimicrobials ^a	<p>Treat for 3-4 days after clinically normal. Uterine evacuation with prostaglandins and tetanus vaccination should also be considered.</p>
Lamb epididymitis	Sheep	<i>H. somni</i> , <i>A. seminis</i> , <i>Corynebacterium pseudotuberculosis</i>	Oxytetracycline	<p><i>Prophylaxis:</i> low levels in feed in situations where rams intensively managed, or injectable long-acting oxytetracycline (IM or SC). Responds poorly to treatment.</p>

(continued)

Table 32.2. Antimicrobial drug selection for common conditions of sheep and goats. (continued)

Condition	Species	Infecting Organism	Suggested Antimicrobial(s)	Comments
Enzootic posthitis	Sheep and goats	<i>C. renale</i> group	Penicillin G; oxytetracycline	Remove from high protein diet and treat locally with antibiotic ointments. May treat systemically for severe cases.
<i>Brucella ovis</i> ram epididymitis	Sheep	<i>Brucella ovis</i>	Oxytetracycline with dihydrostreptomycin	20 mg/kg oxytetracycline at 3-day intervals for 5 treatments & 12.5 mg/kg streptomycin 2X day for 7 days decreases shedding of bacteria & improves semen quality but may not cure. Should consider culling.
Infectious diseases of lambs and kids, systemic				
Enterotoxemia/ Pulpy Kidney	Sheep and goats	<i>C. perfringens</i> type C & D	Oral virginiamycin; penicillin G; or bacitracin	Vaccinate all animals at risk. Withdraw carbohydrate source in diet, give C&D antitoxin and a balanced electrolyte solution (BES) parenterally.
Omphalophlebitis	Sheep and goats	<i>A. pyogenes</i> , <i>E. coli</i> , mixed anaerobes	Penicillin G; broad-spectrum antimicrobials ^a	Antibiotic therapy alone not often effective. Local drainage and treatment and possibly surgical removal should be considered.
Watery Mouth (lambs)	Sheep and goats	Probable <i>E. coli</i> endotoxin	Oral amoxicillin; apramycin; broad-spectrum antimicrobials ^a	Prevention by ensuring clean environment and good colostrum ingestion.
Metabolic acidosis without dehydration (kids)				Watery mouth: early prophylactic treatment with oral antibiotics. Metabolic acidosis: isotonic bicarbonate solutions to correct acid-base deficit followed by balanced electrolyte solution (BES).
Tick Borne Fever (Tick pyaemia)	Sheep	<i>Anaplasma phagocytophilum</i> and/or <i>S. aureus</i>	Long-acting oxytetracycline	At 1 to 3 weeks of age and repeated at 5 to 7 weeks, in addition to dipping with an acaricide at those times.
Erysipelothrix polyarthritis	Sheep	<i>E. rhusiopathiae</i>	Penicillin G	Treat minimum of 3 days.
Infectious diseases of lambs and kids, digestive				
Colibacillosis	Sheep and goats	Enterotoxigenic <i>E. coli</i>	Broad-spectrum antimicrobials parenterally	Appropriate diagnosis is necessary, also treat with BES. Clean environment and adequate colostrum is important. Consider vaccination. Resistance to antimicrobials is common.
<i>Salmonella</i> dysentery	Sheep and goats	<i>S. typhimurium</i> and others	Broad-spectrum antimicrobials ^a	Often poor efficacy due to unpredictable susceptibility patterns. May not eliminate carriers if host adapted species.
Abomasitis/abomasal hemorrhage	Sheep and goats	<i>Clostridium</i> spp.	Oral penicillins	Rarely effective. Should treat symptomatically with antitoxins, nonsteroidal anti-inflammatory drugs, and BES.
Coccidiosis	Sheep and goats	<i>Eimeria</i> sp.	Monensin; lasalocid; decoquinate; salinomycin; amprolium; or sulfonamides	Mixing should be done at a feed mill and all feeds pelleted. Some products can be mixed with salt. Dose varies with feed management. Feed from 2 wks of age until market age. Ionophores toxic to horses and dogs.
Infectious conditions of lamb and kids, respiratory				
Pneumonic pasteurellosis	Sheep and goats	<i>M. haemolytica</i> , <i>P. multocida</i>	Tilmicosin; oxytetracycline; ceftiofur; florfenicol	Long-acting oxytetracycline, tilmicosin or florfenicol can be used as a prophylaxis and during an outbreak therapeutically. Tilmicosin should not be used in goats (therapeutic dose very close to toxic dose). Ceftiofur for daily treatment of affected animals when meat or milk withdrawal is an issue (e.g., market lambs close to slaughter, lactating dairy sheep).
<i>Pasteurella</i> septicemia	Sheep	<i>P. trehalosi</i>	As with <i>M. haemolytica</i>	<i>P. trehalosi</i> shows more resistance and because the disease is peracute, vaccination is recommended for susceptible animals.
Necrotic laryngitis	Sheep and goats	<i>Fusobacterium necrophorum</i>	Penicillin G; oxytetracycline	
<i>Mycoplasma</i> pneumonia	Sheep and goats	<i>M. ovipneumoniae</i> , <i>M. arginini</i>	Oxytetracycline; tylosin	Often seen in conjunction with pasteurellosis (atypical pneumonia) or alone.

Table 32.2. Antimicrobial drug selection for common conditions of sheep and goats. (*continued*)

Condition	Species	Infecting Organism	Suggested Antimicrobial(s)	Comments
<i>Mycoplasma mycoides</i>	Goats	<i>M. mycoides</i> ss. <i>mycoides</i> large colony type	Oxytetracycline; lincomycin; or tylosin	Treatment of peracute septicemia often ineffective. If goat survives, it will probably be a carrier.
Infectious conditions of the integument				
Pinkeye (Infectious keratoconjunctivitis)	Sheep and goats	<i>C. psittaci</i> , <i>M. conjunctivae</i> , <i>R. conjunctivae</i> , <i>Neisseria</i> (least effective).	Spiramycin; oxytetracycline; tiamulin IM	Spiramycin or oxytetracycline repeated days 1, 5 & 10; tiamulin repeated days 1, 3, 6 & 9. Oxytetracycline eye ointment. Conjunctival injection of penicillin ovis
Secondary infection of Contagious Ecthyma (Orf)	Sheep and goats	<i>S. aureus</i>	Tilmicosin; oxytetracycline; ampicillin	May also try local antimicrobials but wear gloves, as is a zoonosis.
Dermatomycosis (lumpy wool)	Sheep	<i>Dermatophilus congolensis</i>	Long acting oxytetracycline	Decrease humidity (ventilation) if possible, and protect from rain. Powder sheep with powdered alum to help prevent reinfection.
Caseous lymphadenitis	Sheep and goats	<i>Corynebacterium pseudotuberculosis</i>	No effective treatment	Although susceptible to penicillin, not effective because of the thick abscess wall. Recommend isolation and drainage with local disinfectants and vaccination of young stock. Cull chronically infected animals.
Infectious conditions of the foot and musculoskeletal system				
Contagious footrot	Sheep and goats	<i>D. nodosus</i> <i>F. necrophorum</i>	Long acting oxytetracycline; penicillin G; tinidazole; spectinomycin; florfenicol	10-20% zinc sulphate with 2% w/v sodium lauryl sulphate or 5% formalin, as a foot bath with or without foot trimming. Must remain in bath one hour. Repeat in 5-7 days. Can use in conjunction with systemic antimicrobials and/or vaccination. Cull chronic non-responders.
Foot scald	Sheep and goats	<i>F. necrophorum</i>	Penicillin G; ampicillin; or long acting oxytetracycline	Zinc sulfate foot bath as above.
Strawberry footrot	Sheep and goats	<i>D. congolensis</i>	As with lumpy wool	Verify that condition is not chorioptic mange.
Polyarthritis	Sheep and goats	<i>Chlamydophila pecorum</i>	Oxytetracycline	
Polyarthritis	Goats	<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> , other <i>Mycoplasma</i> spp.	Oxytetracycline; tylosin	Poor response, may relapse.
Infectious conditions of the mammary gland				
Gangrenous mastitis	Sheep and goats	<i>S. aureus</i> , <i>M. haemolytica</i>	Tilmicosin; broad-spectrum antimicrobials ^a	Gland will be lost if animal survives, so should probably be culled.
Contagious agalactia	Sheep and goats	<i>M. agalactiae</i> , <i>M. mycoides</i> ss. <i>mycoides</i> (goats)	Tetracyclines; tylosin	Probably ineffective, so animal should be culled. Carrier state likely.
Subclinical and clinical mastitis	Sheep and goats	<i>S. aureus</i> , <i>M. haemolytica</i> , environmental streptococci, coagulase negative <i>Staphylococcus</i> spp.	Tilmicosin; cloxacillin; cephalixin benzathine	Dry treatment to be used at the end of lactation in dairy goats or at weaning for prevention of new infections in high-risk sheep flocks. Do not split tubes. Tilmicosin should not be used in goats (therapeutic dose very close to toxic dose).
Infectious conditions of the oral cavity				
Periodontal disease	Sheep	Many species	No effective treatment	Trials examining effect of tetracycline and metronidazole found no protective effect.
Tooth root abscess	Sheep and goats	Many species	Oxytetracycline; florfenicol; broad-spectrum antimicrobials	4-6 weeks of therapy. Consider surgical intervention if antimicrobials fail.

(*continued*)

Table 32.2. Antimicrobial drug selection for common conditions of sheep and goats. (*continued*)

Condition	Species	Infecting Organism	Suggested Antimicrobial(s)	Comments
Actinobacillosis	Sheep	<i>Actinobacillus lignieresii</i>	Sodium iodide	70 mg/kg as 10-20% solution every two weeks for 2-3 doses.
Actinomycosis	Sheep	<i>Actinomyces bovis</i>	Sodium iodide; sulfadimethoxine; isoniazid	As for actinobacillus. Treat for weeks to months.
Infectious conditions of the urinary tract				
Leptospirosis	Sheep and goats	<i>Leptospira interrogans</i>	Dihydrostreptomycin; oxytetracycline	Drugs are potentially nephrotoxic, questionable efficacy.
Cystitis	Sheep and goats	<i>Corynebacterium renale</i> , other species	Broad-spectrum antimicrobials	Therapy should be based on culture and sensitivity and should be given for 10-14 days.
Infectious conditions of the nervous system				
Bacterial meningitis	Sheep and goats	Many species	Broad-spectrum antimicrobials	Anti-inflammatory drugs important. Anti-seizure medications if needed.
Listeriosis	Sheep and goats	<i>L. monocytogenes</i>	Oxytetracycline; Penicillin G	Injectable long-acting formulation. 22,000-44,000 IU/kg IM twice per day. Broad-spectrum antimicrobials include: ampicillin-sulbactam, ceftiofur, fluoroquinolones, trimethoprim-sulfamethazine, or other potentiated sulfonamide combinations.

Table 32.3. Common antimicrobial and coccidiostat drug dosages in sheep and goats.

Drug Preparation	Route	Dose Rate	Units	Frequency (hr)	Withdrawal Time ^b : Meat (days)/Milk (hrs)
Amoxicillin-clavulanic acid	IV, IM	20	mg/kg	8	
Amoxicillin trihydrate	IM	10	mg/kg	8	
Ampicillin sodium	IV,IM	10-20	mg/kg	12	
Ampicillin-sulbactam ^c	IM	10	mg/kg	12-24	
Amprolium	PO, feed ^d , water	10-60	mg/kg	24, for 5 to 21 days (low dose for 21 days for prevention, high dose five days for treatment)	
Ceftiofur sodium	IM	1.1-2.2	mg/kg	24, for 3 days	0/NA
Chlortetracycline	PO	200-400	mg/head	Daily during gestation to prevent infectious abortion	0/NA
Clavulanic acid-amoxicillin	IV, IM	20	mg/kg	8	
Decoquinat	In feed	100	g /ton	Daily in feed for period of coccidiosis risk	
	PO	0.5	mg/kg		
	PO	2	mg/kg	Daily during gestation to prevent <i>T. gondii</i> abortion	
Enrofloxacin (sheep) ^e	IV, IM	5	mg/kg	24	
Erythromycin	IM	3-5	mg/kg	8-12 up to 5 days	3/NA
Florfenicol	IM,SQ	20 (IM); 40 (SQ)	mg/kg	48 (IM) 96 (SQ)	
Lasalocid	In feed	30	gm/ton	Daily in feed for period of coccidiosis risk	
	PO	1	mg/kg		
Lincomycin hydrochloride	IM	10-20	mg/kg	12-24	
Monensin	In feed	11-22	gm/ton	Daily in feed for period of coccidiosis risk	
	PO	1	mg/kg		
	PO	15	mg/kg	Daily during gestation for prevention of <i>T. gondii</i> abortion	
Neomycin sulfate	In feed or water	22	mg/kg	24-for up to 14 days	2 (sheep), 3(goats)/NA
Oxytetracycline hydrochloride	IV, IM	10	mg/kg	12-24	Test milk after 96 hours for one dose and 144 hours for multiple doses
Oxytetracycline long acting	IM	20	mg/kg	48-72	
Penicillin G, potassium or sodium	IV	20,000-40,000	IU/kg	6	
Penicillin G, procaine	IM	20,000-45,000	IU/kg	12	
Salinomycin	In feed	11-16	gm/ton	In feed for period of risk	
Sulfonamides	PO, drinking water	50 (loading 100)	mg/kg	24	
Tilmicosin ^f	SC	10	mg/kg	Single treatment	28 (sheep)
Trimethoprim-sulfonamide	IM	24-30	mg/kg	12-24	
Tylosin	IM	20	mg/kg	12	

^aMany of the drugs listed are not approved for use in sheep and goats in the United States and elsewhere, so that their use constitutes extra-label drug use (ELDU). In some cases, dose recommendations are empirical. Consult the label for details.

^bWithdrawal times are for the United States or Canada or both. Consult labels or global FARAD for withdrawal recommendations.

^cSulbactam-ampicillin given once daily may have too short a half-life to be therapeutically effective in goats and sheep. Plasma levels and post-antibiotic effects for 5 to 12 hrs (longer level for some Gram-positive bacteria).

^dELDU of feed additives is prohibited in the United States.

^eFluoroquinolones are banned from ELDU in food producing animals in the United States.

^fTilmicosin should not be used in goats due to potential toxicity.

four parenteral-use antimicrobial drugs approved in sheep (procaine penicillin G, tetracycline, ceftiofur sodium, and tilmicosin) and only one in goats (ceftiofur sodium). With this very short list of antimicrobials and associated approved indications, the sheep and

goat veterinarian is often forced to use antimicrobial drugs approved for other species in an extra-label manner. The Animal Medicinal Drug Use Clarification Act (AMDUCA) allows this under certain conditions. Any food animal veterinary practitioner should be fa-

miliar with the rules of AMDUCA. For sheep and goats, if the veterinarian in his clinical judgment does not think the labeled drug will work, or there is no labeled antimicrobial for a given indication, a different antimicrobial approved for sheep and goats should be selected first. If an appropriate choice is not available, antimicrobials approved for other food animal species, particularly cattle, should be selected. Antimicrobials approved for non-food animals, and then those approved for humans, should be used only as a last resort. The veterinarian must remember that under AMDUCA, factors such as lower cost, convenience (long-acting formulations, small volumes, good syringability, availability), or short withdrawal times cannot be used as justification for extra-label use.

AMDUCA also states that any extra-label use must be by or on the order of a veterinarian within the context of a veterinarian-client-patient relationship, and must not result in violative residues in food-producing animals. Since few drugs are approved in sheep and goats, meat and milk withdrawal times can be difficult to find. There are publications (Concordet and Toutain, 1997; Riviere, et al., 1998) and websites (www.farad.org) that have information that can help the veterinarian calculate withdrawal times. Their methods commonly use the half-life of a drug, which is in most cases derived in healthy animals. The disposition of the antimicrobial in a diseased animal, especially if the diseased tissue is responsible for elimination of the drug, should be carefully considered, and withdrawal times sufficiently lengthened to account for differences. There are also a few antimicrobials in which blood levels may return to normal, but the drug remains in the tissue for prolonged periods. Examples of this are aminoglycosides in the kidney and tilmicosin in the udder. Although aminoglycosides are not illegal for use in food animals, the American Association of Small Ruminant Practitioners (AASRP) supports a voluntary ban on the use of aminoglycosides in food animals (except for FDA-approved uses) due to the potential for residues. Table 32.4 is included to help the practitioner make informed decisions on drug use and withdrawal time recommendations. For more information on residue avoidance, including help calculating withdrawal times, contact the drug manufacturer, and/or the Food Animal Residue Avoidance Databank (www.farad.org).

The veterinarian must also keep in mind that the extra-label use of some drugs may be prohibited. In

the United States, these are (at time of publication): (1) chloramphenicol; (2) clenbuterol; (3) diethylstilbestrol (DES); (4) dimetridazole; (5) ipronidazole; (6) other nitroimidazoles; (7) furazolidone; (8) nitrofurazone; (9) sulfonamide drugs in lactating dairy cattle (except approved use of sulfadimethoxine, sulfabromomethazine, and sulfaethoxypyridazine); (10) fluoroquinolones; (11) glycopeptides; and (12) phenylbutazone in lactating dairy cattle over 20 months of age.

Good record-keeping practices for both the owner and veterinarian, and individual animal identification are key to preventing violative residues in meat and milk. Good records are also necessary so that injection site reactions are not confused with lesions of caseous lymphadenitis, which is an important contagious disease of sheep and goats. Veterinarians are encouraged to support the United States Department of Agriculture's (USDA) voluntary National Animal Identification System (NAIS).

In addition to FARAD, more information on FDA approved drugs and residue avoidance can be found at www.fda.gov/cvm. More information on the NAIS can be found at www.usda.gov.

Bibliography

- Concordet D, Toutain PL. 1997. The withdrawal time estimation of veterinary drugs: a non-parametric approach. *J Vet Pharm Therap* 20(5):374.
- Elsheikh HA, et al. 1997. Comparative pharmacokinetics of ampicillin trihydrate, gentamicin sulphate and oxytetracycline hydrochloride in Nubian goats and desert sheep. *J Vet Pharm Therap* 20:262.
- Fajt V. Label and extra-label drug use in small ruminants. *Vet Clin N Am: Food Animal Prac* 17(2):403.
- Fariborz S, et al. 2001. Pharmacokinetics and pharmacodynamics of danofloxacin in serum and tissue fluids of goats following intravenous and intramuscular administration. *Am J Vet Res* 62(12):1979.
- Jianzhong S, et al. 2004. Bioavailability of florfenicol in healthy sheep. *J Vet Pharm Therap* 27:163.
- Mordric S, et al. 1998. Pharmacokinetics and pharmacodynamics of tilmicosin in sheep and cattle. *J Vet Pharm Therap* 21:444.
- Pugh DG. 2002. *Sheep and Goat Medicine*. Philadelphia: W.B. Saunders.
- Riviere JE, et al. 1998. Primer on estimating withdrawal times after extra-label drug use. *J Am Vet Med Ass* 213:966.
- USP veterinary pharmaceutical information monographs: antibiotics. 2003. *J Vet Pharm Therap* 26:1.

Table 32.4 Pharmacokinetic data for selected drugs in sheep and goats.

Drug Preparation	Species	Dose ^a (mg/kg)	Volume of Distribution ^b (L/kg)	Clearance (ml/min/kg)	Elimination Half-life ^c (hours)	Protein Binding (%)	Bio-availability (%)	AUC (µg·h/ml)	Peak Serum/Plasma Concentration (µg/ml)	Time to Peak Concentration (hours)
Amikacin ^d	Sheep	7.5	0.2 ± 0.03 (area)	0.7 ± 0.06	1.93 ± 0.27 (initial phase)		70		31.04 ± 3.67	0.5 ± 0
	Sheep	10(IM)			2.42 ± 0.29 (initial phase)					
Amoxicillin	Goats	10	7.15 ± 0.97	57 ± 8	1.58 ± 0.15					
	Goats	10(PO)	10.2 ± 0.96	52.1 ± 4.2	2.23 ± 0.25		63	175 ± 19	0.65 ± 0.05	90 ± 9
	Sheep	10	6.39 ± 0.49	50 ± 4	1.58 ± 0.05					
	Sheep	20(PO)	20.2 ± 2.3	134 ± 14	1.7 ± 0.06		56	103 ± 5	0.39 ± 0.02	173 ± 11
Ampicillin	Goats	10		48.7 ± 5	1.195	13.8		3.59 ± 0.42		
	Sheep	10		4.4 ± 0.32	2.476			39 ± 2.84		
Apramycin	Goats	20	1.36 ± 0.11	11.69 ± 2.3	0.47 ± 0.16 (initial phase)					
	Sheep	20	1.45 ± 0.10	14.14 ± 1.7	1.84 ± 0.19 (initial phase)					
Ceftiofur	Sheep	1.1-2.2			5-6					
	Sheep	1.1-2.2(IM)							4.1-6.2	0.5
Ciprofloxacin	Sheep	2.5(IM)			9.98 ± 2.33				0.14 ± 0.02	5 ± 0.45
Clindamycin	Sheep	20 (IM)							13.8	1
Danofloxacin	Goats	1.25	3.02 ± 0.28		4.67 ± 0.45			2.23 ± 0.11	0.88 ± 0.1	
	Goats	1.25(IM)			4.41 ± 0.23			2.29 ± 0.11	0.33 ± 0.02	0.74 ± 0.15
Enrofloxacin	Sheep	2.5	3.02 ± 0.22	9.17 ± 2.3	3.73 ± 0.44					
	Sheep	2.5(IM)			3.65 ± 0.31				0.78 ± 0.07	1.3 ± 0.11
Erythromycin	Sheep	20(IM)				23				
Florfenicol	Goats	25	0.98 ± 0.9	8.1 ± 2.6	2.3 ± 0.2					
	Goats	25(IM)			3.3 ± 0.75		43 ± 19.6			
	Sheep	20	1.86 ± 0.11	4.3 ± 0.5	18.3 ± 6.76			76.3 ± 9.17		
	Sheep	20(IM)			10.34 ± 1.11	89.04				1.5 ± 0.2
Gentamicin	Goats	5	0.26 ± 0.04 (area)	3.10 ± 0.27	0.96 ± 0.09 (initial phase)					
	Sheep	10	0.24 ± 0.03	1.03 ± 0.15	30.4 ± 18.9 (gamma phase)					
	Goats	5 (IM)			2.37 ± 0.47 (initial phase)	96			33.9 ± 4.37	0.67 ± 0
	Goats	5 (SC)			3.56 ± 0.39 (initial phase)	77			28 ± 3.84	0.66 ± 0
	Sheep	4 (IM)			1.82 (initial phase)	99				
Kanamycin	Goats	10	0.263 ± 0.022	1.5 ± 0.18	1.9 (initial phase)					
	Sheep	10	0.262 ± 0.03 (area)	1.67 ± 0.15	1.8 (initial phase)					
	Sheep	15 (IM)							36.9 ± 8.97	1
Lincomycin	Sheep	20(IM)							12.6	1
Neomycin	Sheep	10	0.304 ± 0.08 (area)	1.52 ± 0.13	1.98 ± 0.5 (initial phase)					
	Sheep	10(IM)			2.68 ± 0.29 (initial phase)		75		17.63 ± 2.27	1.33 ± 0.4
	Sheep	10(SC)			2.82 ± 0.51 (initial phase)		85		18.66 ± 3.05	1 ± 0.32

Table 32.4 Pharmacokinetic data for selected drugs in sheep and goats. (*continued*)

Drug Preparation	Species	Dose ^a (mg/kg)	Volume of Distribution ^b (L/kg)	Clearance (ml/min/kg)	Elimination Half-life ^c (hours)	Protein Binding (%)	Bio-availability (%)	AUC (μg·h/ml)	Peak Serum/Plasma Concentration (μg/ml)	Time to Peak Concentration (hours)
Oxytetracycline	Goats	5		7.3 ± 0.78	3.89			12.08 ± 1.5	0.822 ± 0.221	3.9 ± 2.41
	Sheep	5		4.7 ± 0.4	6.3			18.37 ± 1.7		
Spectinomycin	Sheep	20(IM)	0.307		1.01				53	1
Tilmicosin	Sheep	10(SQ)			34.6 ± 8.1			19.9 ± 3.4		
Tylosin	Goats	15(IM)		6.8	3	38	72.6		2.4	4.2
	Sheep	20		2.1						
	Sheep	20(IM)				38				

^aIntravenous route unless otherwise stated.^bSteady state unless otherwise stated.^cTerminal phase unless otherwise stated.^dA voluntary resolution against the use of aminoglycosides in food-producing animals except as FDA-approved has been instituted by the American Veterinary Medical Association due to prolonged tissue residues following parenteral administration. Systemic absorption is poor when administered orally, but damage to the gut mucosa can lead to increased absorption.

Antimicrobial Drug Use in New World Camelids

Christopher K. Cebra and Margaret L. Cebra

Over the last twenty-five years, the populations of llamas and alpacas, the two domestic and most common species of New World camelids, have increased rapidly in North America, Australia, and most recently, Europe. The combined numbers of these species are roughly seven million in South America (Peru, Chile, Bolivia, and Argentina), three hundred thousand in North America, sixty thousand in Australia, and thirty thousand in Europe. Although llamas enjoyed the earlier popularity in North America and to some degree in Europe, in general, the populations of alpacas are rising more rapidly in all the non-South American countries.

Veterinarians in North America have historically found New World camelids to be frustrating cases, because camelids hide disease signs, physical examination and laboratory evaluation often yield no immediate answers, and little reference information is available. Additionally, sick camelids often have impressive leukogram changes, particularly neutrophilia with or without a left shift. These changes may or may not reflect infectious disease. Stress neutrophilia is common and can lead to nucleated cell counts as high as 50,000 cells/ μ l, as well as moderate increases in band cell counts.

Based on the lack of other definitive indicators and the abnormal leukogram, the decision is often made to try a course of antibiotics. The choice of medication is usually empirical with broad-spectrum coverage desired. This leads to the next frustration: there is a general paucity of disease prevalence data and pharmacokinetic data from New World camelids. Camelids are anatomically and physiologically unique, making extrapolation from other species dangerous. No medications are approved for use in New World camelids, and dosages found in clinical reports can differ from each

other as much as 25-fold. Although camelids are modified ruminants and a source of meat in South America, their "pet" status in North America in general releases veterinarians from the need to avoid medications prohibited from use in food-producing animals and to warn clients of tissue residues, though this must be determined on a case-by-case basis.

Some reasonable dosages can be devised by examining the available information. However, most of these dosages have not been studied scientifically, and the attending veterinarian must assume the responsibility for extra-label drug use and potential adverse effects on the animal. As a general rule, antibiotics appear to have longer elimination half-lives in camelids than in domestic ruminants, potentially prolonging their therapeutic effect but also increasing their toxic risk. This may be due to a lower rate of urine production in camelids, which may increase the half-life of antibiotics excreted primarily through the kidneys (penicillins, aminoglycosides; Lackey et al., 1995). This slower renal excretion in turn may be affected by concurrent fluid treatments, such that camelids in referral hospital situations may be treated similarly to domestic ruminants, whereas camelids treated in the field must be dosed more conservatively. As another general rule, volume of distribution varies tremendously among individual camelids. Higher dosages are generally recommended to avoid subtherapeutic drug concentrations in some camelids. Thus, the most useful antibiotics are those with a high margin of safety.

By far, the antibiotics most commonly reported used in camelids are penicillin G, ceftiofur sodium, and gentamicin sulfate. Often combinations are used to provide broad-spectrum coverage. Surprisingly, no pharmacokinetic studies about penicillin G are available, and most dosages are extrapolated from other

large animal species. Adverse reactions are reported rarely, most commonly after prolonged use, and clinical efficacy appears to be satisfactory. Interestingly, ampicillin (which is excreted by similar renal mechanisms and has a similar half-life as penicillin G in other species) in one small study was shown to have a half-life in llamas two and four times longer than in horses or sheep, respectively, and a volume of distribution at steady state about 50% greater than sheep and about the same size as horses (Christensen et al., 1996). The longer half-life may be the result of low urine production, which could prolong the action of renally excreted medications, and suggests that lower dosages or less frequent dosing intervals of the penicillins in camelids may achieve sufficient therapeutic effect. Administering fluids during the course of antibiotic treatment may negate this effect by enhancing excretion.

Ceftiofur sodium has been studied in both llamas and alpacas, and also has the broadest range of dosages in clinical reports (Christensen et al., 1996; Drew et al., 2004). The two main studies provide conflicting information concerning the volume of distribution at steady state and half-lives, but similar information concerning clearance and area under the curve. Additionally, individual camelids in the larger study had volumes of distribution at steady state that differed by up to 100%. Thus while the larger study reports pharmacokinetic parameters similar to those seen in small ruminants, twice-daily dosing at 2.2 mg/kg intravenously or intramuscularly is recommended to avoid subtherapeutic concentrations in the camelids with greater volumes of distribution. There are currently no scientific reports of using ceftiofur hydrochloride in camelids, but anecdotal reports of its use have revealed no extraordinary rate of complications.

Gentamicin sulfate and the similar compound tobramycin have been studied in llamas and camels (Christensen et al., 1996; Dowling et al., 1996; Hadi et al., 1994; Lackey et al., 1996). Again, conflicting information concerning volume of distribution was generated from the different studies, with the largest study reporting volumes of distribution at steady state that differ up to 150% between animals. Because aminoglycoside antibiotics generally have poor lipid solubility and move into the extracellular space slowly, these differences in volume of distribution could relate to differences in gastric fill and body fat in the individual

camelids. The studies agree on prolonged elimination half-lives of around 3 hours.

As with other species, once-daily dosing of camelids with aminoglycosides has become more popular than more frequent dosing. The rationale of this is to allow trough concentrations to drop below 2.0 µg/ml to prevent nephrotoxicity. Because of camelids' slow elimination of aminoglycosides, this strategy appears to be especially valid. Dosing camelids at 2.5 mg/kg intravenously maintains concentrations above the toxic threshold for at least 6 hours in many camelids (Lackey et al., 1996), which would represent a risk for toxicosis with three-times-a-day dosing. Dosing at 4.0 to 5.0 mg/kg maintains concentrations above the toxic threshold for about 12 hours (Dowling, 1996; Lackey, 1996), and also provides peak concentrations necessary for antimicrobial activity.

Nephrotoxicoses in camelids administered aminoglycosides both once a day and more frequently have been reported in the literature (Hutchison et al., 1993) and anecdotally. The slow elimination, sparse urine production, and extreme variability in volumes of distribution potentially make camelids very susceptible to relative overdose, especially when they are dehydrated or drink insufficient water. Such problems have not been reported (scientifically or anecdotally to the authors) in camelids administered aminoglycosides and concurrent intravenous fluids. Thus, it is especially important to ascertain hydration status before administering aminoglycosides to camelids and during the course of treatment.

Ceftiofur sodium and gentamicin appear to have similar enough pharmacokinetic properties when given intravenously or intramuscularly that the same dosage and dosing frequency may be used regardless of route. Additionally, recent evidence from other species suggests that many antibiotics have comparable absorption from the subcutaneous space. The subcutaneous route has become very popular in camelids for all antibiotics formerly administered intramuscularly due to lack of large muscle masses and ease of administration. Unless a very fast effect is desired or the particular antibiotic is known to cause adverse reactions when given subcutaneously, this route is considered acceptable.

Intravenous sulfa antibiotics and sulfa-trimethoprim combinations have been studied and used on clinical cases (Chakwenya et al., 2002; Christensen et al., 1996; Junkins et al., 2003; Snook et al., 2002). At reduced,

metabolically-scaled doses, sulfadimethoxine in llamas has a higher volume of distribution at steady state and a longer half-life than in cattle, but may fail to reach therapeutic blood concentrations (Junkins et al., 2003). Trimethoprim-sulfamethoxazole combinations have rapid elimination in llamas (Christensen et al., 1996) and especially alpacas (Chakwenya et al., 2002) and hence may not remain above therapeutic concentrations at conventional dosages and dosing rates. It is interesting to note that trimethoprim and sulfa antibiotics are both eliminated at least in part through hepatic biotransformation, but that like antibiotics cleared exclusively through renal mechanisms, they also have shorter half-lives than in other species.

Intravenous and subcutaneous enrofloxacin, and intravenous and intramuscular oxytetracycline and florfenicol, have also been studied in llamas and alpacas. Intravenous florfenicol had a lower volume of distribution than in sheep, goats, or camels, and a slightly longer half-life than in sheep or goats (Ali et al., 2003; Christensen et al., 2001). Intravenous oxytetracycline in llamas had a similar volume of distribution to camels, but a much longer half-life (Oukessou et al., 1992). Alpacas had a larger volume of oxytetracycline distribution, but a similar half-life to camels. Enrofloxacin reaches therapeutic concentrations after intravenous or subcutaneous dosing, but published information regarding its half-life is conflicting. (Christensen et al., 2001; Gandolf et al., 2004).

Oral antibiotics have been studied less extensively. Adult camelids should be expected to have similar problems with absorption as adult ruminants. Trimethoprim and sulfa antibiotics appear to have poor absorption at ruminant dosages and cannot be recommended for systemic disorders (Chakwenya et al., 2002; Junkins et al., 2003; Snook et al., 2002). Oral tetracycline and chloramphenicol use have also been reported, but no pharmacokinetic studies have been performed. Oral enrofloxacin has a 29.3% bioavailability, and reaches therapeutic concentrations after dosing at 10 mg/kg (Gandolf et al., 2004). Oral antibiotics might be more useful in pre-ruminant camelids, but this usage has not been investigated.

One topic that has received attention in recent years is the dosing difference between llamas and alpacas. Pharmacokinetic studies have approximately followed species popularity, with the earlier studies concerning llamas and more recent studies concerning alpacas,

though the greater availability of llamas as test subjects has influenced the continued appearance of studies on llamas. Few studies have compared the two species, which recently have been declared members of separate genera.

Glucose studies indicate that adult alpacas have an extracellular fluid compartment approximately 37% larger than adult llamas (Cebra et al., 2004a). This is similar to the difference in volume of distribution for oxytetracycline found in one study (Christensen et al., 2001), whereas the volume of distribution for ceftiofur is reported to be 2.5 to 3 times larger in alpacas than llamas (Drew et al., 2004; Christensen et al., 1996).

A physical basis for the difference in volumes of distribution is found in the contributions of various organs to whole body weight. The full gastric viscera make up approximately 4% more of whole body weight in llamas than in alpacas, meaning alpacas generally have proportionally more soft tissue and extracellular fluid (Cebra et al., 2004b). Very lipophilic compounds such as florfenicol distribute across these gastric membranes and hence have similar volumes of distribution in llamas and alpacas, whereas hydrophilic compounds with good distribution through the extracellular fluid compartment would be expected to spread over a larger volume in alpacas than in llamas. Dosage adjustment may be necessary, as has been demonstrated with oxytetracycline. Aminoglycoside antibiotics, which, although hydrophilic, appear to diffuse more slowly out of the vascular compartment, would be less affected by this and hence should not be dosed higher in alpacas.

The same argument can be used to adjust dosages for younger camelids. Glucose studies suggest that unweaned llama crias between 2 and 4 weeks of age have an extracellular fluid compartment approximately 30% larger than adult llamas (Cebra et al., 2005). Unfortunately, the importance of this difference in antimicrobial dosages has not been investigated.

Compared with many other common domestic species, much less information is available concerning the frequency and importance of bacterial isolates. We have tried to compile what has been seen at Oregon State University and what is available in the scientific literature (Table 33.1). Sufficient data were not available to derive meaningful sensitivity conclusions. Since many of these bacteria are opportunists, they would likely have similar sensitivity profiles to isolates from other species. Of particular note here are the α he-

Table 33.1. Bacterial isolates from camelid lesions.

	Wound or Superficial Lesion	Tooth Root Abscess	Female Repro Tract	Sepsis Adult	Sepsis Cria	Soft Tissue Abscess	Myositis
Gram-negative							
<i>Escherichia coli</i>	3 (1)	2	25	11 (3)	7 (4)		1
<i>Pseudomonas</i> spp.	2		7	1 (2)	2 (1)		
<i>Actinobacillus</i> spp.				4 (1)	1 (1)		
<i>Salmonella</i> spp.				2 (2)	(2)		
<i>Fusobacterium</i>	2	7 (6)				2	
<i>Bacteroides</i> spp.	2 (1)	6 (10)		1 (3)			1 (1)
Other Gram-negatives	1		2	1	2 (1)	1 (1)	
Gram-positive							
<i>Staphylococcus</i> spp. (coagulase -)	1	(1)	3	1			
<i>Staphylococcus</i> spp. (coagulase +)	5			1			
Non-hemolytic streptococci				(5)	(1)		
α -Hemolytic streptococci	(2)	1		3 (5)	1		
β -Hemolytic streptococci	3		1	5 (1)	3	2 (1)	2
<i>Enterococcus</i> spp.			5	2	1		(1)
<i>Rhodococcus equi</i>						(1)	
<i>Clostridium</i> spp.		(2)		6 (4)		(1)	1 (2)
<i>Actinomyces</i> spp.	1 (1)	7 (8)		1		7 (1)	
<i>Peptostreptococcus</i>	1	2 (5)					
<i>Arcanobacterium pyogenes</i>			2			1	
<i>Listeria monocytogenes</i>				1 (4)	(1)		
<i>Bacillus</i> spp.			2	(1)			

Source: Oregon State University Veterinary Diagnostic Laboratory and selected scientific publications. (Non-OSU cases in parentheses.)

molytic streptococci, which often are resistant to penicillin and may be the cause of some treatment failures.

As stated above, specific localized syndromes (such as bacterial pneumonia or enteritis) are rare, so aside from the chronic, focal infections, most bacterial diseases have been grouped together as septic conditions. These animals usually present with general systemic signs including fever, inappetance, obtundation, and weakness, but may also have specific signs referable to the affected organs. As a general conclusion, the relative equality between Gram-negative and Gram-positive isolates, both from wounds and in camelids with sepsis, supports the initial use of broad-spectrum antibiotics. Combinations of an aminoglycoside with a β -lactam antibiotic or ceftiofur alone are most common. Other single medications such as oxytetracycline, enrofloxacin, or florfenicol may be useful in some situations. Collection and culture of pertinent body fluids (blood, peritoneal fluid, pleural fluid, cerebrospinal fluid, urine, feces, aspirates, etc.) may yield information about specific pathogens and allow refinement of antibiotic selection.

Female reproductive tract infections frequently involve Gram-negative enteric bacteria and are frequently treated with gentamicin infusions, whereas tooth root abscesses and other tissue abscesses more frequently involve Gram-positive or anaerobic bacteria and are most commonly treated with long courses (20 to 60 days) of penicillin, ceftiofur, or florfenicol. The camelid blood parasite, *Mycoplasma haemolamae*, is most commonly treated with long-acting oxytetracycline preparations. Chapters on the individual antibiotics should be consulted for additional information concerning use and specific contraindications. Dosage recommendations are presented in Table 33.2.

Fungal diseases are reported in camelids, but no drug studies have been conducted. Systemic mycoses include aspergillosis, coccidioidomycosis, cryptococcosis, histoplasmosis, and mucormycosis. Dermal or superficial mycoses include candidiasis, ringworm, and entomophthoromycosis. Systemic medication use has been extremely limited, with all dosages extrapolated from ruminants.

Table 33.2. Common antimicrobial drug dosages in adult New World camelids.^a

Drug Preparation	Dose	Dose Interval (h)	Route of Administration
β-Lactams^{b,c,d}			
Benzyl penicillins:			
Penicillin G (Na, K)	22,000-44,000 IU/kg	6	IV
Penicillin G (procaine)	22,000-44,000 IU/kg	12-24	IM or SC
Aminobenzyl penicillins:			
Ampicillin sodium	10-20 mg/kg	8-12	IV or IM
Ampicillin trihydrate	10-20 mg/kg	12-24	IM or SC
3rd-generation cephalosporin:			
Ceftiofur	2.2-4.4 mg/kg	12-24	IV, IM, or SC
Aminoglycosides ^e			
Amikacin	18-21 mg/kg	24	IV, IM, or SC
Gentamicin	4.4-6.6 mg/kg	24	IV, IM, or SC
Fluoroquinolones			
Enrofloxacin ^f	5 mg/kg	12-24	IV, IM, or SC
Tetracyclines ^{b,c}			
Oxytetracycline (100 mg/ml)	10 mg/kg	12-24	IV
Oxytetracycline (200mg/ml)	20 mg/kg	24-72	IM or SC
Other			
Florfenicol	20 mg/kg	48	IM or SC
Metronidazole ^g	15-25 mg/kg	8-12	PO or per rectum
Trimethoprim-sulfamethoxazole	18 mg/kg (combined)	12	IV, IM, or SC

^aAlthough these medications and dosages have been used repeatedly for camelid patients in referral hospitals in North America, pharmacokinetic and safety data are lacking for use in camelids. Use caution and monitor carefully for adverse reactions or toxic effects.

^bHigher dosages and/or shorter intervals for more severe infections.

^cHigher dosages and/or shorter intervals may be indicated in alpacas or llamas with lower gastric fill or body fat.

^dHigher dosages and/or shorter dosing intervals may be indicated in young camelids.

^eLarge differences in volume of distribution between individual camelids and risk of nephrotoxicosis with overdose support caution in the use of this medication at any dose, especially the higher dosages, and especially in camelids with decreased urine production.

^fShould not be used in young, growing camelids because of the risk of arthropathy.

^gOral dosing in juvenile and adult camelids will affect the gastric microbial population; administer per rectum.

Bibliography

- Ali BH, et al. 2003. Comparative plasma pharmacokinetics and tolerance of florfenicol following intramuscular and intravenous administration to camels, sheep and goats. *Vet Res Commun* 27:475.
- Cebra CK, et al. 2004a. Meta-analysis of glucose tolerance in llamas and alpacas. *Proc 4th European Symposium South American Camelids*.
- Cebra CK, et al. 2004b. Determination of organ weights in llamas and alpacas. *Proc 4th European Symposium South American Camelids*.
- Cebra CK, et al. 2005. Evaluation of glucose tolerance and insulin sensitivity in llama crias. *Am J Vet Res* 66:1013.
- Chakwenya J, et al. 2002. Pharmacokinetics and bioavailability of trimethoprim-sulfamethoxazole in alpacas. *J Vet Pharmacol Ther* 25:321.
- Christensen JM, et al. 1996. The disposition of five therapeutically important antimicrobial agents in llamas. *J Vet Pharmacol Ther* 19:431.
- Christensen JM, et al. 2001. Comparative metabolism of oxytetracycline and florfenicol in the llama and alpaca. Unpublished data.
- Dowling PM, et al. 1996. Pharmacokinetics of gentamicin in llamas. *J Vet Pharmacol Ther* 19:161.
- Drew ML, et al. 2004. Pharmacokinetics of ceftiofur in llamas and alpacas. *J Vet Pharmacol Ther* 27:13.
- Gandolf AR, et al. 2004. Addressing the need for oral antibiotic treatment options in camelids. Alpaca Research Foundation <http://www.alpacaresearchfoundation.org/>.
- Hadi AA, et al. 1994. Pharmacokinetics of tobramycin in the camel. *J Vet Pharmacol Ther* 17:48.
- Hutchison JM, et al. 1993. Acute renal failure in the llama (*Llama glama*). *Cornell Vet* 83:39.
- Junkins K, et al. 2003. Disposition of sulfadimethoxine in male llamas (*Llama glama*) after single intravenous and oral administrations. *J Zoo Wildl Med* 34:9.
- Lackey MN, et al. 1995. Urinary indices in llamas fed different diets. *Am J Vet Res* 56:859.

Lackey MN, et al. 1996. Single intravenous and multiple dose pharmacokinetics of gentamicin in healthy llamas. *Am J Vet Res* 57:1193.

Oukessou M, et al. 1992. Pharmacokinetics and local tolerance of a long-acting oxytetracycline formulation in camels. *Am J Vet Res* 53:1658.

Snook CS, et al. 2002. Plasma concentrations of trimethoprim and sulfamethoxazole in llamas after orogastric administration. *J Vet Pharmacol Ther* 25:38.

Antimicrobial Drug Use in Swine

Robert M. Friendship

The modern swine industry has moved to reduce the use of antimicrobial drugs through various husbandry and management improvements. New vaccines, segregated production practices which allow the use of all-in/all-out pig flow, and greater attention to biosecurity have all resulted in improved pig health and less reliance on antimicrobials. The industry goal is to produce safe, uncontaminated meat in a cost-effective manner, with consideration for animal welfare and consumer and environmental issues. Many of the major pork producing countries have introduced programs to educate farmers regarding proper drug use, including the recording of all treatments and the identification of animals medicated, to ensure that appropriate withdrawal times are observed (Lewis-Jones, 1998; Stefan, 1998). In addition, tighter government regulations and increased testing are being instituted. There is greater monitoring of antimicrobial resistance, particularly in the case of zoonotic pathogens such as *Salmonella* spp.

The vast majority of antimicrobial drugs used on swine farms are incorporated into feed for the purpose of growth promotion (2–50mg/ton of feed) or for sub-therapeutic use (200 g or less/ton of feed). The swine industry continues to use more in-feed antibiotics than other livestock industries. The use of antibiotics to improve growth rate and feed efficiency is discussed elsewhere (Chapter 24). The present chapter will deal primarily with antimicrobial drugs as therapeutic agents and will be presented on the basis of body systems (Table 34.1). Dosages for commonly used antimicrobial drugs are presented in Table 34.2.

General Considerations of Antimicrobial Treatment

Antimicrobial drugs are generally administered to a group of pigs rather than to individuals because of the large numbers of animals held in a pen or a barn. Individual treatment is very labor-intensive and can be stressful for the pigs. The exception is the breeding herd, where individual treatment is the most common approach.

A treatment program should depend on the antibiotic susceptibility pattern of the pathogenic organism and the established MICs of the antibiotics being considered (Chapter 2). The choice of antimicrobial drug and method of administration also depend on in-depth knowledge of the specific pig farm, including the ability of the farm workers to carry out the treatment regimen, and the success or failure of different antibiotics used previously on the farm. Most health problems on pig farms are caused by a complex interaction of environment, management and husbandry factors, and concurrent infection with multiple disease-causing organisms. It is important for the success of any medication program to be combined with good husbandry practices.

Route of Administration

Antimicrobials are usually delivered through a carrier (feed or water). However, parenteral injection often results in the best response. Administration of medication to individual pigs per os is limited to nursing piglets.

The delivery of drugs via the feed or water is less stressful for the pig and easier to apply than parenteral treatment, but tends to be more costly because all animals in the group receive medication, rather than just

Table 34.1. Antimicrobial drug selection in swine infection.

Diagnosis	Causative Agent	Comments	Suggested Drugs
Enteric			
Clostridial enteritis	<i>Clostridium perfringens</i> Types A and C	Treatment of affected piglets is not effective after clinical signs have been observed. Treat sows to reduce shedding.	Bacitracin in sow feed Ampicillin to piglets (PO)
Coccidiosis	<i>Isospora suis</i>	Piglets must be treated prior to clinical signs. No approved drugs in Canada or USA.	Toltrazuril (PO) Amprolium (PO)
Colibacillosis	<i>Escherichia coli</i>	Resistance is common. Electrolytes may be useful.	Gentamicin (PO) Trimethoprim-sulfa (IM) Apramycin (water)
Colitis	<i>Brachyspira pilosicoli</i>	Disease is often mild and may respond to a feed change.	Tiamulin (water, feed) Lincomycin (water, feed) Tylosin (feed)
Intestinal parasitism	<i>Ascaris suum</i> <i>Hyostromylus rubidus</i> <i>Oesophagostomum</i> spp. <i>Trichuris suis</i> <i>Strongyloides ransomi</i>	Confinement housing and good hygiene reduce problem. Sows should be treated before farrowing to prevent infecting piglets.	Ivermectin (SC, feed) Doramectin (IM) Fenbendazole (feed) Pyrantel tartrate (feed)
Proliferative enteropathy	<i>Lawsonia intracellularis</i>	Good hygiene can minimize disease effects. Treatment must be given early in the course of the disease. Prophylactic indication is often used in high risk groups.	Piperazine (water, feed) Tylosin (feed) Lincomycin (feed) Tiamulin (feed)
Salmonellosis	<i>Salmonella enterica</i>	Oral antibiotics prolong shedding and may promote resistance.	
Swine dysentery	<i>Brachyspira hyodysenteriae</i>	Resistance against older drugs is common. Treatment for extended time after clinical signs disappear is necessary.	Tiamulin (water, feed, IM) Lincomycin (water, feed)
Respiratory			
Enzootic pneumonia	<i>Mycoplasma hyopneumoniae</i> and secondaries	Environmental and housing stresses are important. Parenteral treatment is often necessary to achieve therapeutic levels.	Oxytetracycline (IM) Trimethoprim-sulfa (IM) Ceftiofur (IM) Tulathromycin (IM) Florfenicol (IM) Tiamulin (feed, water). Tilmicosin (feed)
Pleuropneumonia	<i>Actinobacillus pleuropneumoniae</i>	Many strains resistant to tetracyclines and tylosin. Feed and water treatment is a problem because of decreased consumption when animal is sick.	Trimethoprim-sulfa (IM) Ceftiofur (IM) Tulathromycin (IM) Florfenicol (IM) Tiamulin (feed, water). Tilmicosin (feed)
Progressive atrophic rhinitis	<i>Bordetella bronchiseptica</i> and toxigenic strains of <i>Pasteurella multocida</i>	Responds to environmental and management changes, and vaccination programs.	Oxytetracycline (IM) Sulfamethazine (feed)
Lungworm	<i>Metastrongylus</i> spp.	Life cycle requires earthworm as an intermediate host.	Fenbendazole (feed) Ivermectin (feed, SC)
Polysystemic			
Actinobacillus septicemia	<i>Actinobacillus suis</i>	Emerging disease of multi-site production nurseries. Must treat promptly.	Ampicillin (IM) Trimethoprim-sulfa (IM) Ceftiofur (IM)
Erysipelas	<i>Erysipelothrix rhusiopathiae</i>	Treat for several days to prevent chronic form of disease.	Procaine penicillin G (IM)
Glasser's disease	<i>Haemophilus parasuis</i>	Resistance to penicillin can be a problem. High doses given promptly.	Procaine penicillin G (IM) Ceftiofur (IM) Trimethoprim-sulfa (IM)
Mycoplasma septicemia	<i>Mycoplasma hyorhinis</i>	High dosages administered parenterally as soon as clinical signs observed, but results are often poor.	Lincomycin (IM) Tylosin (IM) Tiamulin (IM)

Table 34.1. Antimicrobial drug selection in swine infection. (*continued*)

Diagnosis	Causative Agent	Comments	Suggested Drugs
Salmonellosis	<i>Salmonella</i> Choleraesuis	Vigorous treatment early.	Ceftiofur IM Trimethoprim-sulfa (IM) Ampicillin (IM)
Neurological			
Edema disease	<i>Escherichia coli</i>	Treatment is generally unsuccessful if clinical signs have developed.	Apramycin (water)
Otitis media	<i>Staphylococcus</i> spp. <i>Streptococcus</i> spp. <i>Arcanobacterium pyogenes</i> and others	Abscessation may make treatment difficult.	Trimethoprim-sulfa (IM) Ampicillin (IM)
Streptococcal meningitis	<i>Streptococcus suis</i>	Separate sick pigs into hospital pens and provide water. Pigs may not be able to walk, so nursing care is essential.	Procaine penicillin G (IM) Ampicillin (IM) Trimethoprim-sulfa (IM) Ceftiofur (IM) Procaine penicillin G (IM)
Tetanus	<i>Clostridium tetani</i>	Prognosis is poor. Antitoxin and relaxant could be used.	
Musculoskeletal			
Footrot	<i>Fusobacterium necrophorum</i>	Topical agents such as copper sulfate are useful.	Procaine penicillin G (IM) Tetracyclines (IM)
Mycoplasma arthritis	<i>Mycoplasma hyosynoviae</i>	Injectable antibiotics plus corticosteroids for rapid improvement.	Lincomycin (IM) Tylosin (IM) Tiamulin (IM)
Neonatal polyarthritis	<i>Streptococcus</i> spp. <i>Staphylococcus</i> spp. <i>Escherichia coli</i> <i>Arcanobacterium pyogenes</i>	Vigorous treatment early is necessary to prevent chronic arthritis problems.	Procaine penicillin G (IM) Lincomycin (IM) Tylosin (IM)
Suppurative arthritis	<i>Arcanobacterium pyogenes</i> and possibly <i>Staphylococcus</i> and <i>Streptococcus</i>	Treatment is generally ineffective.	Procaine penicillin G (IM) Lincomycin (IM)
Urinary			
Cystitis and pyelonephritis	<i>Actinobaculum suis</i> and possibly others	Relapses are common.	Procaine penicillin G (IM)
Kidney worm	<i>Stephanurus dentatus</i>	Unlikely to occur in confinement reared pigs.	Ivermectin (SC, feed) Doramectin (IM) Fenbendazole (feed)
Reproductive			
Leptospirosis	<i>Leptospira</i> spp.	Antibiotics may not eliminate carriers.	Tetracycline (IM, feed) Streptomycin (IM)
Mastitis and/or metritis	Generally Gram-negative bacteria	Cross-foster piglets or provide additional milk. Treatment depends on microorganism sensitivity.	Ampicillin (IM) Ceftiofur (IM) Trimethoprim-sulfa (IM)
Cardiovascular			
Eperythrozoonosis	<i>Mycoplasma haemosuis</i> (<i>Eperythrozoon suis</i>)	Control lice to reduce spread. Tetracycline (IM, feed). Arsenical (feed, water)	
Skin			
Exudative epidermitis (greasy pig disease)	<i>Staphylococcus hyicus</i>	Treat wounds with topical skin disinfectants. May see resistance to penicillin.	Procaine penicillin G (IM) Trimethoprim-sulfa (IM)
Lice	<i>Haematopinus suis</i>	Eradication requires 2 treatments 14-20 days apart to all pigs.	Ivermectin (SC, feed) Doramectin (IM)
Mange	<i>Sarcoptes scabiei</i>	Eradication requires 2 treatments 14-20 days apart to all pigs.	Ivermectin (SC, feed) Doramectin (IM)

Table 34.2. Common antimicrobial drug dosages in pigs.

Drug	Dose(mg/kg), Interval ^a	Feed (g/ton, US)	Water (mg/L)
Ampicillin	6.5, 24h		
Apramycin	10-20 (oral), 12-24h	150	100
Arsanilic acid		50-100	
Bacitracin		250	
Carbadox		50	
Ceftiofur	3-10, 24h		
Clavulanate-amoxicillin	11-13 (oral), 24h		
Erythromycin	2-20, 12-24h		
Florfenicol	15, 24h		
Gentamicin	5 (oral), 24h		
Lincomycin	10, 24h	100-220	33
Neomycin	10 (oral), 6h	140	70-100
Procaine penicillin	20,000-45,000 IU/kg, 24h		
Salinomycin		400	
Spectinomycin (use with lincomycin)			100
Streptomycin	25, 24h		
Sulfamethazine	24 (oral), 24h	400-2,000	80-130
Tetracyclines ^b	10-20, 24h	200-800	55-110
Tetracycline (long-acting)	20, 48h		
Tiamulin	2-11, 24h	200	50
Tilmicosin		200-400	
Trimethoprim-sulfadoxine	16, 12h		
Tulathromycin	2.5, 24h		
Tylosin	9, 12-24h	40-100	80
Virginiamycin		100	

^aAdminister IM unless otherwise specified.^bTetracycline, oxytetracycline, chlortetracycline.

the sick pigs. Oral medication reduces injection-related side effects, including anaphylactic shock, lesions from broken needles, abscesses, and tissue damage at the injection site.

Intramuscular injections should be made on the lateral side of the neck near the base of the ear. Injection into the back leg is discouraged because of the risk of scarring the valuable ham and possibly causing damage to the sciatic nerve. Needles are more likely to be broken when injections are made in the leg as opposed to the neck.

Subcutaneous injections can be made in the neck region under the loose skin behind the ear in older pigs. In young animals, SC injections can be given in the flap of skin inside the flank of the abdominal wall. Intravenous injections are usually given in the ear vein.

Although the most common method of administering antimicrobials is via the feed, there are a number of disadvantages with this method. The technique is

wasteful in that healthy pigs receive medication along with the sick animals, and animals may receive medication for a longer period of time than is necessary. The sick animals often have poor appetites and therefore may not consume sufficient quantities of drug to achieve therapeutic levels in their bodies. In addition, many antibiotics are poorly absorbed from the gastrointestinal tract or are destroyed by stomach acid. There is often a delay in treatment because unmedicated feed must be eaten or removed from the feeding system before medicated feed reaches the pigs. In-feed medication is most appropriate when combating gastrointestinal disease and when it is necessary to medicate over a period of several weeks in order to control a problem. In recent years there has been a move to liquid feeding, often using fermentation. These diets tend to be acidic (often below pH 4.5) and therefore it is necessary to consider the stability of antimicrobials when incorporated into these acidic diets.

Water medication is generally a more rapid method

of treating a group of sick pigs than feed medication. There is also the advantage that sick pigs often continue to drink when they will not eat, although with certain respiratory diseases a marked decrease in water consumption is often noted. A major disadvantage is that not all medication is water soluble, and some medications that are sold as water additives can settle out of suspension or clog water nipples.

Medication is administered via water by using either an in-line water proportioner containing a concentrated stock solution or a large tank filled with water that is medicated at the appropriate level, so that the water from this tank replaces the usual water supply.

Pigs will drink approximately 8–10% of their body weight per day, but this tends to change as the animal grows. A young pig is expected to drink close to 10% and an older pig in the late stages of the grower-finisher period may only consume 6–7% of its body weight. Environmental temperature, feed formulation, flow rate, and design of the watering device all affect water consumption or disappearance (Kolb, 1996). As a rule of thumb when calculating how much medication will be needed to treat a pen of pigs, one can estimate the total weight of the pigs and plan on using 5L medicated water per 60 kg body weight daily. Proportions can be inaccurate and distribution throughout the barn can be uneven. Careful attention is required to ensure that pigs receive adequate amounts of drugs.

Food Safety

Pork producers are aware of the need for domestic and international consumer confidence in their products. The use of antimicrobial drugs in the production of pork has created two issues of consumer concern. First, there are fears that pork from animals treated with antimicrobial drugs will contain harmful residues (see Chapter 25). The second major concern is the emergence of antibiotic-resistant bacteria from the use of antimicrobials on pig farms.

In North America, the majority of pigs receive medication at some stage in their lives. Medication of starter rations for newly weaned pigs is routine on most farms in countries that do not restrict such practices through legislation. There is an association between the use of medicated feeds and antimicrobial resistance (Dunlop et al., 1998). This is a concern for veterinarians choosing a drug to treat a production

disease in the pig herd, but also a concern for human health. The impact of antimicrobial use on farms leading to antimicrobial resistance in human pathogens is not known. In 1999 the European Union banned the use of growth promoting antimicrobials and as a result antimicrobial use on Danish pig farms was reduced by over 50%. This has been associated with a dramatic reduction in enterococci resistant to the growth promoters but a minor increase in the cost of production of swine (World Health Organization, 2002). Monitoring programs have been developed in many countries (for example in the United States, the National Antimicrobial Resistance Monitoring System, NARMS) to document emerging resistance problems. In swine, expanded-spectrum cephalosporin resistance in multi-drug resistant *E. coli* and *Salmonella* serotypes (the *cmv-2* gene encoding expanded-spectrum cephalosporin resistance) is an example of a serious resistance concern that warrants attention.

There are also problems associated with the diminishing choice of drugs to treat swine diseases. For example, there is a high frequency of resistance to multiple antimicrobial drugs in porcine enterotoxigenic *E. coli*. Furthermore, there is some evidence of the emergence of genetic linkage between resistance and elevated virulence in new serotypes (Noamani et al., 2003), such that use of antimicrobial drugs may not only maintain resistance but also lead to more virulent bacteria.

Antimicrobial residues, particularly sulfonamides, continue to be a problem in the swine industry. As a result of increased awareness and extensive testing, the prevalence of sulfamethazine residues in pork has decreased but has not been eliminated. Inadvertent contamination of feeds during preparation or storage and exposure of pigs to contaminated manure have been implicated in cases of sulfamethazine residue problems (Whipple et al., 1980). However, in most cases, antimicrobial residues in pork are caused by farmers' failure to observe appropriate withdrawal times.

The use of medication in an off-label manner is at least partly responsible for some of the difficulties that farmers face in knowing when it is safe to ship an animal to market. For extra-label drug use, withdrawal information may be obtained from the manufacturer or in some cases from national or international databases such as, in the United States, the Food Animal Residue Avoidance Databank (FARAD).

To improve the quality and safety of pork, various countries have instituted education/certification programs for pig farmers (for example in the United States, Pork Quality Assurance, PQA). The use of on-farm logbooks for recording all antimicrobial drug usage has been advocated as a practical method of increasing a farmer's awareness of antibiotic use and improving adherence to withdrawal times (Elbers et al., 1990). Along with accurate and complete drug recording, there should be periodic visits by a veterinarian to review the farm's medication programs and help ensure that drugs are being used in a safe and efficacious manner.

Considerations in Treating Swine Respiratory Disease

The economic impact of respiratory diseases in swine production is great. The causes of respiratory disease are infectious and widespread. Often the cause is a complex of viral and bacterial etiological agents and the disease expression is influenced by housing and management factors. The term porcine respiratory disease complex (PRDC) has been used to describe particularly severe enzootic pneumonia in the late grower-finisher stage, where viral components can often be identified together with *Mycoplasma hyopneumoniae* and other bacteria (Dee, 1996).

Viral diseases of particular importance are porcine reproductive and respiratory syndrome (PRRS), swine influenza (SI) and post-weaning multisystemic wasting syndrome (PMWS). The PRRS virus and *M. hyopneumoniae* act synergistically to create a more severe pneumonia with a longer duration than either organism causes independently (Thacker et al., 1998). The bacterial respiratory disease agents can be categorized under the following three headings (Stevenson, 1998): (1) primary inhaled pulmonary pathogens such as *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, and *Bordetella bronchiseptica*; (2) secondary pulmonary pathogens that require tissue or cell damage by other bacteria or viruses in order to overcome the respiratory defense, such as *Pasteurella multocida*, *Mycoplasma hyorhinis*, *Streptococcus suis*, and *Hemophilus parasuis*; and (3) blood-borne pulmonary pathogens. One approach to eradication is depopulation of the entire herd and repopulation with specific-pathogen-free (SPF) pigs. In production systems

where weaning occurs at an early age and the nursery is managed in an all-in/all-out manner on a separate site away from other age groups of swine, primary respiratory pathogens can be eliminated from the grower-finisher herd (Clark et al., 1991; Fenwick et al., 1996).

Progressive atrophic rhinitis (PAR) is no longer a significant swine disease because of the widespread use of all-in/all-out management, effective vaccination, and strategic medication programs. In herds experiencing a severe outbreak of PAR, suckling piglets can be medicated by strategic injections of antibacterial drugs 4–8 times before weaning (De Jong, 1999). Potentiated sulfonamides and oxytetracycline are the antimicrobials of choice for treating PAR.

Treatment of enzootic pneumonia by mass medication is generally unrewarding. In the face of a severe outbreak, the choice of medication should take into account the sensitivity of the secondary organisms as well as that of *M. hyopneumoniae*. Achieving sufficiently high drug levels in the lung tissue is difficult unless parenteral treatment is performed. Preventive medication programs based on pulse dosing in the feed are commonly employed. Often these programs use combinations of tetracycline with tiamulin or lincomycin (Kavanaugh, 1994; Desrosiers, 1997). Prevention of enzootic pneumonia depends on good management and environment. Vaccination has proven to be effective. The incidence and severity of pneumonia is greatly increased by factors such as continuous flow of animals, large numbers of pigs in a single air space, overcrowding, a high degree of mixing of pigs within the barn, and introducing pigs from multiple sources. In addition, environmental factors such as high levels of dust or manure gases, drafts, and cold stress lead to increased severity of pneumonia.

Pleuropneumonia has become less of a problem in most swine rearing areas in recent years. When serious outbreaks occur, pigs can die as early as 6–12 hours post-infection. It has been shown that feed and water consumption during an outbreak can decrease by 85% (Pijpers et al., 1990). Therefore under these circumstances medication must be started early, and parenteral injection is the method of choice. Many isolates of *A. pleuropneumoniae* have developed resistance to tetracyclines and penicillin. Ceftiofur, trimethoprim-sulfadiazine, and tiamulin are appropriate alternative therapeutic agents.

Considerations in Treating Swine Enteric Disease

Enteric diseases are less of a problem in recent years owing to improved control of pig flow allowing for thorough cleaning, and the use of slatted flooring and overall better hygiene.

There are a number of causes of neonatal diarrhea including *E. coli*; *Clostridium perfringens*, *Isospora suis*, coronavirus, and rotavirus. Of these pathogens, *E. coli* is the most common cause of piglet disease and colibacillosis is the only disease of suckling pigs that readily responds to antibiotic treatment. Culture and susceptibility should be performed to ensure the appropriate antibiotic is used and the diagnosis is accurate. Commonly, oral or intramuscular treatment of individual scouring piglets with a single treatment of 5 mg gentamicin is sufficient to alleviate the problem.

Post-weaning colibacillosis is possibly a more important problem than the neonatal disease and tends to be more difficult to treat. Mass medication, typically in the water, using apramycin or neomycin is a common approach to an outbreak situation. Where available, colistin (polymyxin E) has been shown to be useful against enteropathogenic *E. coli* (Lanza, 1998). High-level zinc oxide (2000-3000 ppm) in the starter feed for a couple of weeks has been shown to reduce postweaning *E. coli* diarrhea (Melin et al., 1996). Other approaches, including vaccination; feeding probiotics, prebiotics, or immunoglobulins; and acidification of water or feed have been used but with mixed results.

Several bacterial enteric diseases of the young growing pig are economically significant. Swine dysentery caused by *Brachyspira hyodysenteriae*, and colonic spirochetosis caused by *Brachyspira pilosicoli* are diseases of the pig's large intestine that cause a mucoid diarrhea. Swine dysentery is the more severe of the two diseases and is often associated with bloody diarrhea and relatively high mortality rates. *Salmonella* also tends to cause disease of the large bowel and may be difficult to distinguish from these spirochete diseases. Porcine proliferative enteropathy or ileitis is caused by *Lawsonia intracellularis* and is a common cause of ill-thrift or even sudden death in pigs from the nursery to young adults.

Of these enteric diseases, swine dysentery is the one which has responded to management changes and has greatly diminished in importance. Swine dysentery

can be eradicated from herds with strategic medication and sanitation (Glock, 1997). Pigs acutely infected with swine dysentery must be treated by injection or water medication. Initial treatment must be followed by medication in the feed for several weeks to prevent recurrence. Tiamulin is an effective choice of drug to treat and eradicate swine dysentery (Molnar, 1996). Valnemulin, a pleuromutilin derivative related to tiamulin, has also been shown to be highly effective against *B. hyodysenteriae* and *B. pilosicoli* (Wade-West and Ripley, 1998).

Although there have been few trials to evaluate antimicrobials against *B. pilosicoli*, it is generally accepted that drugs with efficacy against swine dysentery will be successful. This disease appears to be associated with non-infectious factors such as feed processing, feed ingredients, and other endogenous microflora, making interpretation of clinical trials somewhat difficult (Duhamel et al., 1996).

Porcine proliferative enteropathy has not decreased in significance despite changes in husbandry such as segregated early weaning. In general, macrolides, lincosamides, pleuromutilins, tetracyclines, and carbadox appear to be effective in treating or preventing ileitis. Pigs showing clinical signs of chronic disease may not respond to treatment because the disease has progressed too far before intervention has begun. However, the acute disease does respond to prompt injections of tylosin or other effective drugs, and the inclusion of therapeutic levels of antimicrobials in the feed at an early stage of the chronic disease can greatly reduce the severity of the lesions (McOrist et al., 1997; Winkelman, 1997). Vaccination is available and may prove to be an effective alternative to the use of antimicrobials.

Salmonellosis occurs as a septicemic disease caused by *Salmonella* Choleraesuis or as an enteric problem associated with various serovars of *Salmonella enterica*, particularly Typhimurium. The prevalence of *S. Choleraesuis* seems to have greatly declined during the past 20 years and is seldom a problem. On the other hand, *Salmonella* Typhimurium has greatly increased in importance, both as a cause of clinical swine disease and from a public health standpoint. Antibiotics are contraindicated for the treatment of enteric salmonellosis. Whereas they might tend to improve the clinical expression, they do not reduce shedding. It is likely that antibiotic therapy encourages the development of resistance.

Considerations in Treating Swine Parasitic Diseases

In modern, intensive, confinement swine operations, pigs are usually infected with only a few worm species at low levels (Roepstorff and Jorsal, 1989). Under conditions of good hygiene and management, the regular application of anthelmintics may be of little or no additional effect (Roepstorff, 1997). In herds where internal parasitism persists at an economically significant level, ascariasis is generally the most important problem. The migration of *Ascaris suum* larvae through the liver results in scarring and rejection of livers at the packing plant. In addition, larval migration in the lungs can exacerbate viral or bacterial pneumonia. The most common control method is the regular use of anthelmintics at strategic times in the production cycle. Generally, these treatments are given via the feed to sows prior to farrowing to eliminate shedding of eggs in the farrowing room, where piglets would become exposed. Weaned pigs are often medicated once or twice during the grower stage. Most of the modern anthelmintics available to pig farmers are effective in the control of ascarids. Ivermectin or doramectin are commonly used if mange is present on the farm in order to control both external and internal parasitism. If trichuriasis is a problem, fenbendazole rather than ivermectin should be used.

External parasitism caused by mange and lice is no longer a significant problem on most farms owing to the effectiveness of drugs and the change in management toward segregating production groups and using all-in/all-out pig flow. Failure to control mange or lice is generally a result of a lack of understanding of the epidemiology of the organisms or apathy on the part of the herdsman (Dobson and Davies, 1992). Eradication of these parasites can be accomplished by treating the entire herd at one time with an effective drug such as ivermectin. Generally a follow-up treatment 10 to 14 days after the initial treatment is recommended to ensure that mites and lice hatching from eggs will be killed and that no animals were accidentally missed or underdosed.

Coccidiosis remains an important problem in swine production. Where available, toltrazuril has proven to be an effective method of control. Elsewhere control tends to rely on the use of flooring that allows good sanitation and general supportive therapy for scouring pigs. Poultry coccidiostats such as amprolium and decoquinate have been used to some extent.

Bibliography

- Cargill C, Davies PR. 2006. External parasites. In: Straw BE, et al., eds. *Diseases of Swine*, 9th ed. Ames, IA: Blackwell Publishing, p875.
- Clark LK, et al. 1991. The effect of all-in/all-out management on pigs from a herd with enzootic pneumonia. *Vet Med* 86:946.
- Dee SA. 1996. The porcine respiratory disease complex: are subpopulations important? *Swine Health Prod* 4:147.
- DeJong MF. 2006. Progressive and nonprogressive atrophic rhinitis. In: Straw BE, et al., eds. *Diseases of Swine*, 9th ed. Ames, IA: Blackwell Publishing, p577.
- Desrosiers R. 1997. Diagnosis and control of swine respiratory diseases. *Am Assoc Swine Pract*, 333.
- Duhamel GE, et al. 1996. Prevalence of porcine spirochetosis in a multiple-site production system and antimicrobial sensitivity patterns of *Serpulina pilosicoli*. *Am Assoc Vet Lab Diagn*, 45.
- Dunlop RH, et al. 1998. Associations among antimicrobial drug treatments and antimicrobial resistance of fecal *Escherichia coli* of swine on 34 farrow-to-finish farms in Ontario, Canada. *Prev Vet Med* 34:2.
- Elbers ARW, et al. 1990. Log-book recording on pig finishing farms in an integrated quality control (IQC) project: (1) use of drugs and vaccines. *Tijdschr Diergeneeskde* 115:2.
- Fenwick B, et al. 1996. Serological validation of the utility of early weaning in preventing sow to piglet transmission of *Actinobacillus pleuropneumoniae*: production of disease free pigs from infected breeding herds. *Int Pig Vet Soc*, 482.
- Glock RD. 1997. Elimination and control of swine dysentery. *Am Assoc Swine Pract*, 379.
- Kavenaugh NT. 1994. The effect of pulse medication with a combination of tiamulin and oxytetracycline on the performance of fattening pigs in a herd infected with enzootic pneumonia. *Irish Vet J* 47:58.
- Kolb JR. 1996. Vaccination and medication via drinking water. *Compend Contin Educ Pract Vet* 18:575.
- Lanza I. 1998. Control of infectious enteric diseases of swine. *Int Pig Vet Soc*, 79.
- Lewis-Jones CA. 1998. Quality assurance assessment. *Pig J* 41:99.
- McOrist S, et al. 1997. Oral administration of tylosin phosphate for treatment and prevention of proliferative enteropathy in pigs. *Am J Vet Res* 48:136.
- Melin L, et al. 1996. The influence of zinc oxide on the intestinal microflora of piglets at weaning. *Int Pig Vet Soc*, 465.
- Molnar L. 1996. Sensitivity of strains of *Serpulina hyodysenteriae* isolated in Hungary to chemotherapeutic drugs. *Vet Rec* 138:158.
- Naomani BN, et al. 2003. Virulence genes of O149 enterotoxigenic *Escherichia coli* from outbreaks of postweaning diarrhea in pigs. *Vet Microbiol* 97:87.
- Pijpers A, et al. 1990. Feed and water consumption in pigs following an *Actinobacillus pleuropneumoniae* challenge. *Int Pig Vet Soc* p39.
- Roepstorff A. 1997. Helminth surveillance as a prerequisite for anthelmintic treatment in intensive sow herds. *Vet Parasitol* 73:139.

- Roepstorff A, Jorsal SE. 1989. Prevalence of helminth infections in swine in Denmark. *Vet Parasitol* 33:231.
- Stefan GE. 1998. How the residue monitoring program will change. *Proc A D Leman Conf*, 197.
- Stevenson GW. 1998. Bacterial pneumonia in swine. *Int Pig Vet Soc*, 351.
- Thacker E, et al. 1998. Mycoplasma and PRRSU interactions—their possible roles in PRDC. *Am Assoc Swine Pract*, 351.
- Wade-West G, Ripley RH. 1998. The treatment of naturally occurring swine dysentery with Encor under field conditions. *Int Pig Vet Soc*, 138.
- Whipple DM, et al. 1980. Tissue residue depletion and recycling of sulfamethazine in swine. *J Am Vet Med Assoc* 177:1348.
- Winkelman N. 1997. Enteric clinical disease—'back to basics'. *Am Assoc Swine Pract*, 353.
- World Health Organization. 2000. Global principles for the containment of antimicrobial resistance in animals intended for food. Geneva, Switzerland. <http://www.who.int/en/>.

Antimicrobial Drug Use in Poultry

Charles L. Hofacre

The commercial poultry industry, whether it is fully integrated or not, is generally a very intensive animal agriculture system. For example, one poultry house can contain as many as 100,000 commercial layers. This means that disease prevention is the major focus for a poultry veterinarian. However, under those circumstances when husbandry and biosecurity procedures fail to prevent the introduction of a disease agent, appropriate antimicrobial therapy becomes necessary not only to avoid economic losses but also to prevent pain and suffering in these birds. When the poultry veterinarian decides that birds should be treated with an antimicrobial, the next decision is which drug and route of administration is most appropriate. The choice of antimicrobial drug is often limited by regulatory approvals, the cost of the drug vs. the level of disease, and the safe duration of withdrawal prior to harvest to eliminate drug residues in meat or eggs for human consumption.

Under current husbandry conditions in the poultry industry, the segregation and medication of individual sick birds is not feasible. In addition, antimicrobial interventions must be administered early in the course of disease. Bacterial infections in birds tend to progress rapidly, and there is frequently a very short time from initial infection to death. In addition, birds appear quite adept at producing inflammatory responses, but poor at resolving the products of such responses. Birds tend to obscure clinical signs of disease. Spotting the very early, subtle signs of an infection in individuals in a flock of 10,000–100,000 birds is both important and problematic. Treatment of all individuals in contact and at high risk of exposure (i.e., the entire flock) is the only practical approach to disease outbreaks in large flocks. Thus the decision to treat a “sick flock” of birds means veterinarians will be administer-

ing antimicrobials not only to the sick birds, but also to all birds in that flock that have been or will be exposed to the disease agent. In making this decision to treat the “sick flock”, the poultry veterinarian must also decide, based on clinical judgment, whether the flock to be treated means the entire farm or only the house containing the most clinically affected birds. A rapidly spreading disease may necessitate prophylactic treatment of all houses on the farm.

Antimicrobial drug use in poultry can be divided into two broad use categories: therapeutic and growth promotant. However, both categories are therapeutic, in that the drugs are killing or inhibiting the growth of disease-causing agents (bacteria or coccidia). Use of antimicrobials for either category has the potential to select for bacterial strains that are resistant to the antimicrobials used. The antimicrobial drugs in the growth promotant category are in some instances the same antimicrobials used in therapy; however, the dose level of the drug administered is usually less in growth promotion. Growth promotant antimicrobial drugs are administered only in the birds’ feed. The decision to use growth promotant antimicrobials in commercial poultry is primarily based on economic factors—improvement of body weight gain, feed efficiency, and growth rate—that provide economic benefits greater than the cost of the drug. Since there are a limited number of therapeutic antimicrobials approved for use in commercial poultry, in most countries, these are rarely used as growth promotants. For example, penicillin or tetracyclines are rarely used as growth promotants even though there may be regulatory approval for this use.

The fully integrated nature of the commercial poultry industry allows poultry-producing companies to implement bird husbandry practices that impact a

large proportion of the meat supply for the consumer. It is imperative that preventive health programs, as well as appropriate therapeutic strategies, are practiced not only to avoid disease in the birds but also to prevent introduction of potential human foodborne pathogens. Therefore, when birds must be treated with antimicrobials, not only effectiveness against the disease-causing agent but also the impact on foodborne pathogens enter into the decision-making process of the poultry veterinarian. The ever-increasing consumer pressure on food retailers to limit the use of antimicrobial agents in food-producing animals limits the choices for therapy by poultry veterinarians and the means by which they can ensure that healthy birds are brought to market.

Physiological, Pathological, and Husbandry Conditions Affecting the Outcome of Antimicrobial Therapy

Escherichia coli is the leading cause of disease-related economic loss for the poultry industry throughout the world (Barnes et al., 2003). Therefore, it is the primary bacterial infection that therapeutic antimicrobials are used to treat. In most instances, these *E. coli* infections are secondary infections following a primary viral or environmental insult (Glisson, 1998). Thus therapeutic antimicrobials in commercial poultry are almost always used to relieve the suffering of the sick birds, control morbidity and mortality, and minimize the financial impact of the disease on bird performance until the primary insult can be identified and controlled or eliminated. The use of therapeutic antimicrobials also decreases the public health risk associated with slaughtering birds from sick flocks. Poultry that are sick eat greater amounts of bedding material (litter), resulting in higher rates of *Salmonella* and *Campylobacter* spp. in their intestinal tracts (Corrier et al., 1999). Also, Russell (2003) found that birds from flocks having higher airsacculitis condemnation had higher levels of *E. coli* and *Campylobacter*.

The choice of therapeutic antibiotics available to treat respiratory infections caused by *E. coli* is limited (Glisson and Hofacre, 2004). The economic value of the individual animal is so low that it is cost-prohibitive to individually dose each bird in a house, eliminating the option of parenterally administering drugs such as aminoglycosides and cephalosporins. An

additional argument against parental administration is that the stress on birds when individually handled could result in a more rapid progression of the disease. Since sick birds continue to drink, therapeutic antibiotics labeled for use in drinking water are most often used. The tetracyclines, enrofloxacin, and the sulfonamides are the primary drugs used to treat *E. coli* airsacculitis. It can be speculated that this limited choice of antibiotics has, over 30 years, resulted in selection pressure on *E. coli* in the commercial poultry environment, resulting in the high levels of sulfonamide (93%) and tetracycline (87%) resistance in clinical *E. coli* isolates observed in many diagnostic laboratories (Zhao et al., 2005).

Flock treatment (Table 35.1) is the method of choice, with drinking water and feed the primary means of delivering antimicrobials to commercial poultry. When birds become sick, there is a significant reduction in consumption of both feed and drinking water. The decline in drinking water consumption is usually much less than that of feed. Therefore, the route of choice for administering antimicrobials in the early stages of a disease is usually by the birds' drinking water. If therapy lasts more than 5–7 days, then the veterinarian may choose to have the antimicrobial drug added to the birds' feed, if an approved feed-grade product is available. This change to feed can be based upon the flock beginning to recover and eating more. In general, feed-grade antimicrobials are also less expensive than water soluble ones, and are therefore often preferred when a suitable drug with a clinically effective inclusion rate is available.

Another consideration when selecting the appropriate antimicrobial is the ambient temperature, since poultry have limited means of eliminating heat from their bodies. In large part, they cool themselves by drinking water; therefore, water consumption increases significantly as the ambient temperature increases. This both affects dosage calculation and makes it possible for birds to consume a toxic dose when a drug is administered in drinking water. This is especially important when considering the use of sulfonamides, since the therapeutic dose is close to the level that can result in toxic side effects (Goren et al., 1984). Fortunately, bacterial diseases in general tend to be less common in hot weather.

Lighting schedules and feed programs can also strongly influence both feed and water consumption. Laying hens will begin to eat when the lights are

Table 35.1. US FDA approved therapeutic antimicrobials for poultry.

Antibiotic	Egg Layer Chickens			Broiler Chickens			Turkeys		
	Feed	Drinking Water	Growth Promotant	Feed	Drinking Water	Growth Promotant	Feed	Drinking Water	Growth Promotant
Bacitracin	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Bambermycin	No	No	No	Yes	No	Yes	Yes	No	Yes
Chlortetracycline	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Erythromycin	Yes	No	No	Yes	Yes	No	Yes	Yes	No
Enrofloxacin	No	No	No	No	Yes	No	No	Yes	No
Lincomycin	No	No	No	Yes	Yes	Yes	No	Yes	Yes
Neomycin	Yes*	No	No	Yes*	Yes	No	Yes*	Yes	No
Novobiocin	No	No	No	Yes	No	No	Yes	No	No
Oxytetracycline	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Penicillin	No	No	No	Yes	No	Yes	Yes	Yes	Yes
Spectinomycin	No	No	No	No	Yes	No	No	Yes	No
Streptomycin	No	No	No	No	Yes	No	No	No	No
Sulfonamides**	No	No	No	Yes	Yes	No	Yes	Yes	No
Tetracycline	No	No	No	No	Yes	No	No	Yes	No
Tylosin	Yes	No	Yes	Yes	No	Yes	No	No	No
Virginiamycin	No	No	No	Yes	No	Yes	Yes	No	Yes

Note: Gentamicin and ceftiofur are labeled for day-old chickens and turkeys by subcutaneous injection only.

*Approved for use in combination with oxytetracycline.

**Rofenaid, a potentiated sulfonamide with ormetoprim, is approved for use in feed for broiler chickens, layers less than 16 weeks old, and turkeys.

turned on and then consume water after eating. Broiler chickens and turkeys that are on continuous light eat and drink intermittently at 3–4 hour intervals. Maximal water intake in replacement breeders under feed restriction occurs for only a few hours after feeding.

It should be apparent that administering antimicrobials to poultry based solely on concentration of the active ingredient in the drinking water and ignoring the above described physiological, pathological, and husbandry conditions can lead to highly inaccurate dosing. The most accurate method is to calculate the dose based upon the total body weight of birds in the house, and then include that dose in the volume of water or feed the birds are expected to consume during each dosing interval.

Pharmacological Considerations in Antimicrobial Therapy of Poultry

The success of an antimicrobial medication is dependent upon many interacting factors, including pharmacodynamics (drug interaction with bacterial

cells), pharmacokinetics (drug absorption, distribution, excretion) and the components of the host immune system (Chapters 4, 5). The activity of an antimicrobial agent against a particular microbe is often expressed as the minimal inhibitory concentration (MIC) (Chapter 2). When interpreting antimicrobial susceptibility information, the poultry veterinarian must keep in mind that this is an *in vitro* test that does not take into consideration whether the drug can reach the site of infection or whether the drug is bacteriostatic or bactericidal for the microbe. It should also be remembered that the MIC is usually performed by the laboratory on only one isolate, and as was previously stated, many infections of poultry are secondary, so a “sick flock” is often affected by multiple isolates that can have a wide range of MICs. Also, some MIC breakpoint criteria in veterinary medicine are not uniform worldwide and are often based on standards for human medicine (Chapter 2). Additionally, pharmacokinetic data determined in mammals are not always applicable to poultry because birds have higher body temperatures, higher metabolic rates, and a shorter alimentary tract, which often results in lower plasma half-life times for medications.

This frequently leaves the poultry veterinarian with an antimicrobial therapy decision based upon clinical judgment from previous similar cases rather than on the uncertain available science. The primary criterion for measuring success of treatment under poultry industry conditions is reduction of morbidity and mortality. Other important parameters include return to regular water and feed consumption, normal growth rate, and normal egg production.

The immune status of the flock must also be taken into account when deciding which antimicrobial agent to use and the dose rate. For example, chickens experiencing an *E. coli* airsacculitis outbreak secondary to immune suppression by infectious bursal disease virus should be treated with a bacteriocidal drug such as enrofloxacin. However, a bacteriostatic drug such as oxytetracycline may be more effective in treating *E. coli* airsacculitis that is secondary to a respiratory infection by an infectious bronchitis virus.

Pharmacological Characteristics of Poultry Antimicrobials

Beta-lactams (Cephalosporins and Penicillins)

Despite years of use, penicillin G is still an effective antimicrobial for Gram-positive bacterial infections in poultry. The one Gram-negative bacterium routinely treated with penicillin is *Pasteurella multocida*. Penicillin G is formulated for both drinking water and feed administration. The broader spectrum beta-lactams, such as ampicillin, are more effective for Gram-negative infections such as *E. coli* airsacculitis. There is only one cephalosporin, ceftiofur, approved in the US for use in poultry. Since it is so poorly absorbed orally, ceftiofur is only approved for subcutaneous injection. It is commonly administered with Marek's disease vaccine to day-old chicks (Kinney and Robles, 1994). Although it is not an approved method in the US, ceftiofur can also be administered safely with Marek's disease vaccine by *in ovo* injection at 18 days of incubation. The need for ceftiofur, a third-generation cephalosporin, should be assessed against the risk of selecting for resistance to this important group of drugs, including the danger of selection of multidrug-resistant *Salmonella* carrying the *bla_{CMY2}* resistance gene, since such isolates would also be resistant to ceftriaxone, a drug used to treat salmonellosis in people (Chapter 8).

Polypeptides

Bacitracin is the only poultry-approved polypeptide antimicrobial. It is poorly absorbed when administered orally in poultry. However, bacitracin is a very effective antimicrobial for treatment of Gram-positive enteric infections such as necrotic enteritis caused by *Clostridium perfringens* (Hofacre, 1998). It is available in both drinking water and feed grades, with the feed-grade form commonly used to improve the birds' performance.

Aminoglycosides and Aminocyclitols

Three aminoglycosides are used in poultry: gentamicin, neomycin, and streptomycin. Because aminoglycosides are poorly absorbed from the gastrointestinal tract when administered orally, their primary usage has been by subcutaneous injection. However, neomycin is commonly used to treat enteric infections and is administered in either feed or water. Gentamicin is the most widely used aminoglycoside, and it is used primarily as a day-old subcutaneous or *in ovo* injection in chickens or turkeys (McCapes, 1976; Vernimb, 1977). Because gentamicin is a highly basic compound, it can damage cell-associated Marek's disease vaccine if used at too high a dose (greater than 0.2 mg/chick) or improperly mixed with the vaccine (Kinney and Robles, 1994). Streptomycin is partially absorbed from the intestines and therefore can be used to treat systemic *E. coli* infections.

Spectinomycin and hygromycin are the two poultry-approved aminocyclitols. Hygromycin is administered in the feed as an anthelmintic. Spectinomycin is a relatively safe antimicrobial in poultry that is orally absorbed when administered in the drinking water. It is highly efficacious for *E. coli* infections when combined with lincomycin. Rapid development of resistance and higher cost limit the use of spectinomycin.

Macrolides and Lincosamides

Erythromycin is most frequently used in poultry to treat *Staphylococcus aureus* arthritis. Tylosin has been one of the most effective antimicrobials to treat mycoplasma infections in laying hens to restore egg production and reduce transovarial transmission (Bradbury et al., 1994). The macrolides are only bacteriostatic, which may be one reason that their use will not entirely eliminate *Mycoplasma* spp. infections from a flock. Tiamulin, a semi-synthetic macrolide

available outside the US for poultry, has excellent efficacy against *Mycoplasma* spp. infections (Laber and Schutze, 1977).

The only poultry-approved lincosamide is lincomycin. Although it is absorbed with oral administration in feed or water, lincomycin is primarily used to treat enteric infections in poultry. It is commonly used to treat *Clostridium perfringens*-induced necrotic enteritis and also to enhance poultry performance.

Chloramphenicol and Florfenicol

The potential for fatal aplastic anemia in humans resulted in the prohibition of chloramphenicol in animals grown for human consumption throughout most of the world (Chapter 15). However, the closely related antimicrobial florfenicol lacks the chemical group that causes anemia, so it is used to treat *E. coli* airsacculitis infections in poultry (Afifi and El-Sooud, 1997). There has been one report of severe muscle degeneration in broiler chickens treated concurrently with both lasalocid and chloramphenicol (Perelman et al., 1986); there is no information as to whether this may occur with florfenicol.

Tetracyclines

The tetracyclines are the most widely used antimicrobials in poultry. This is largely due to their broad-spectrum of activity (*Mycoplasma*, Gram-positive and Gram-negative bacteria) and wide margin of safety. The tetracyclines are administered in both feed and water. Since they are only slightly soluble in water at pH 7.0, concurrent use of citric acid greatly enhances their absorption from the gastrointestinal tract (Clary et al., 1981). Tetracyclines are readily chelated in the intestine by divalent cations such as calcium or magnesium, resulting in reduced absorption. Therefore the dosage of tetracyclines to laying hens on a high-calcium diet should be increased. After administration is complete, it is recommended to include additional calcium in the diet to improve egg shell thickness and make up for calcium lost to tetracycline binding and intestinal excretion. Tetracyclines are also incompatible with concurrently administered oral electrolytes.

Three tetracyclines most commonly used in poultry are chlortetracycline, oxytetracycline, and tetracycline. It appears that any differences in clinical efficacy of these tetracyclines are primarily because of differences in absorption, drug distribution, or rate of excretion, and not because of differences in bacterial susceptibil-

ity, since there is complete cross-resistance (Chapter 14). It should be remembered that *E. coli* airsacculitis is a secondary infection and even though the *E. coli* isolate selected for susceptibility testing demonstrates resistance to tetracyclines, therapy of a flock of poultry with a tetracycline may still be successful in reducing the clinical signs. This might be because, for example, tetracycline will inhibit *Mycoplasma* that predisposed to *E. coli* infection.

Sulfonamides

The sulfonamides are broad-spectrum antimicrobials widely used to treat or prevent coccidial infections in poultry. There are a wide variety of sulfonamides available for both feed and water administration. Sulfonamides are more soluble in an alkaline pH. Therefore, when administering sulfonamides in acidic water, it may be necessary to raise the pH of the water with household ammonia if the drug precipitates in the bulk tank or stock solution.

The use of sulfonamides has been limited in poultry because of their narrow margin of safety and problems of tissue residues at harvest. Toxic effects of sulfonamides include bone marrow suppression, thrombocytopenia, and depression of the lymphoid and immune function of birds (Chapter 16). This is frequently manifest as pale, almost yellow colored bone marrow and petechial or ecchymotic hemorrhages on the breast, thigh, and leg muscles. The most frequent toxic side-effect of sulfonamide therapy in laying hens is a decline in egg production and egg shell quality (loss of brown pigment). The ambient temperature must be noted when deciding to administer a sulfonamide in the drinking water because as the birds become warmer, they will increase their rate of water consumption to cool themselves. This can quickly result in toxicity. There is one potentiated sulfonamide in the US (sulfadimethoxine/ormetoprim) approved for use in the feed. The combination of these drugs allows for a therapeutic dose at a much lower level of each product, lessening the risk of overdose toxicity.

The other major "side effect" of administering the sulfonamides in poultry is the presence of violative residues of the drug in meat or eggs. Poultry are highly coprophagic and the drug is excreted in the urine and feces; therefore, recycling by coprophagy can result in residues of the drug beyond the stated withdrawal time (Gupta and Sud, 1978). A poultry veterinarian prescribing a sulfonamide should include additional

withdrawal days to ensure there is adequate time for drug clearance (greater than 7–10 days) prior to harvest of meat or eggs.

Quinolones and Fluoroquinolones

Many of the quinolones, such as naladixic acid or oxolinic acid, have been used in poultry to treat primarily Gram-negative bacterial infections. However, when these compounds are used, resistance in the bacterial population in these flocks develops quickly and can eventually result in more rapid resistance developing to the fluoroquinolones (Glisson, 1997). Therefore poultry veterinarians should not recommend the use of these older quinolones in commercial poultry.

The fluoroquinolones are some of the most effective antimicrobial compounds developed for use in poultry. These compounds are highly effective against Gram-positive, Gram-negative, and *Mycoplasma* infections. It was shown that one of the fluoroquinolones, enrofloxacin, eliminated a *Mycoplasma gallisepticum* infection in laying hens (Stanley et al., 2001). However, the fluoroquinolones are less effective against anaerobic bacteria such as *Clostridium perfringens*.

The fluoroquinolones have a wide margin of safety in poultry. They are rapidly absorbed from the gastrointestinal tract, reaching peak blood levels within 1 to 2 hours after ingestion. The long half-life of the fluoroquinolones results in a significant post-antibiotic effect. This gives the poultry veterinarian the opportunity to administer the fluoroquinolones by a “pulsed dose” method in the drinking water (Charleston et al., 1998), which takes advantage of concentration-dependent killing to help prevent the emergence of resistance (Chapter 17). Rapid development of resistance to fluoroquinolones is a significant problem (Chapter 17), and has resulted in resistance occurring in *Campylobacter jejuni*. This issue is discussed in Chapter 3.

The presence of multivalent cations in the intestine or in the drinking water (water hardness ≥ 1300 ppm) will adversely influence the absorption of the fluoroquinolone (Sumano et al., 2004). Therefore it is not recommended to concurrently administer electrolytes with a fluoroquinolone.

Ionophores

The primary usage of ionophore antimicrobials in poultry is to prevent coccidial infections. However, they have been shown to have activity against Gram-

positive bacteria, especially anaerobes such as *Clostridium perfringens*.

Since the ionophores function by altering cell permeability of both prokaryotic and eukaryotic cells, the toxic side effects in poultry are reluctance to move and paralysis. This is caused by muscle weakness resulting from passive transport of potassium out of the cells, with calcium entering. Ionophore toxicity is more severe in adult birds and especially turkeys, even at a safe therapeutic dose for young chickens.

Novobiocin

Novobiocin is rarely used in commercial poultry. It is primarily used to treat juvenile pullets or hens early in the laying house for *Staphylococcus aureus* arthritis. Novobiocin is poorly water soluble, and so must be administered in the feed. High cost is a major reason for its limited use.

Nitrofurans

The nitrofuran antimicrobials have been removed from systemic use in poultry in much of the world because of their carcinogenic potential. They are broad-spectrum antimicrobials that were at one time commonly added to poultry starter feed to reduce the effects of egg-transmitted *Salmonella* infections in the first two weeks of life. In poultry, nitrofuran toxicity results in congestive cardiomyopathy (ascites) or central nervous system signs (Zaman et al., 1995).

Antimicrobial Drug Application under Commercial Poultry Conditions

Since commercial poultry are food animals, the choice of antimicrobials to treat the most common bacterial diseases is limited (Table 35.2). The decision to treat is usually made prior to the results of culture and susceptibility testing. Oral treatment of poultry requires that the drug be stable and be uniformly distributed in either feed or water. When a feed-based antimicrobial is prescribed, the time required for the medicated feed to be manufactured, transported, and delivered through the feeding system at the farm must be taken into account.

Administering the antimicrobial in the drinking water allows for more rapid treatment. The volume of water consumed in 24 hours by the birds in the house to be treated must first be determined. Freshly med-

Table 35.2. Antimicrobial treatment options in poultry.

Disease/Bacterial Species	Antimicrobial																
	Bactracin	Bambermycin	Ceftiofur	Chlortetracycline	Enrofloxacin	Erythromycin	Gentamicin	Lincomycin	Neomycin	Novobiocin	Oxytetracycline	Penicillin	Spectinomycin	Streptomycin	Sulfonamide	Tylosin	Virginiamycin
Arthritis/ <i>Staphylococcus aureus</i>				X		X				X	X	X	X	X			
Chronic Respiratory Disease (CRD)/ <i>Mycoplasma</i> spp.				X	X*						X		X			X	
Colibacillosis/ <i>Escherichia coli</i>				X	X						X		X	X	X		
Erysipelas/ <i>Erysipelothrix rhusiopathiae</i>												X					
Fowl Cholera/ <i>Pasteurella multocida</i>				X	X*						X	X	X	X	X		
Fowl Coryza/ <i>Haemophilus paragallinarum</i>				X		X					X			X	X	X	
Gangrenous dermatitis/ <i>Clostridium</i> spp.				X		X					X	X		X		X	
Necrotic enteritis/ <i>Clostridium perfringens</i>	X	X						X	X			X	X	X		X	X
Omphalitis/ <i>Pseudomonas</i> spp., Enterobacteriaceae			X	X			X				X		X	X	X		
Salmonellosis/ <i>Salmonella</i> spp.				X			X				X			X	X		

Information based on published data and clinical experience; use may be extra-label.

*Extra-label use of enrofloxacin is illegal in the US.

icated solutions should be prepared every day. Drinking water medication is usually administered by either a bulk tank or a water proportioner. Bulk tanks contain 500–2000 liters, and all of the medication for a given tank's volume of water is added to it. A water proportioner is a device that meters the antimicrobial from a highly concentrated stock solution into the drinking water to achieve the appropriate concentration.

The daily dose should always be calculated based on the body weight of a sample of birds and not on water consumption. Dosing based on water consumption can result in a toxic overdose if the ambient temperature increases, or the amount of drug may drop below the MIC of the bacteria being treated if the ambient temperature declines. Additionally, younger birds consume more water daily per unit of body weight than older birds. Dosing at a constant rate per liter of drinking water can result in overdosing of young chicks or underdosing of older birds. In addition, hens producing eggs will drink more per unit of weight than non-laying hens or roosters. Approved daily dosages are shown in Table 35.3.

In situations where the birds' water consumption is limited, a short, intensive treatment with certain antimicrobials may be administered as a pulse dose (Charleston et al., 1998). This method should only be used with bactericidal antimicrobials and those with a wide margin of safety. Pulse dosing requires that all of

the medication to be administered for a 24-hour period is mixed into the water the birds will consume in 6 hours.

Responsible Use of Antimicrobials in Poultry

The responsible use of antimicrobial drugs in poultry which produce meat and eggs for human consumption is based upon good professional judgment, laboratory results, medical knowledge, and information about the flock to be treated. When a flock of commercial poultry begins to exhibit signs of illness, the birds should be physically examined (antemortem and post-mortem). If possible, bacterial cultures should be taken to confirm the clinical diagnosis and to determine the susceptibility of the isolate to the chosen antimicrobial. The potential for rapid spread of disease on a poultry farm often necessitates treatment prior to the results of bacterial culture and sensitivity testing. When laboratory results are completed, the poultry veterinarian must use clinical judgment to decide between continuation or change in therapy. Also, a flock will usually have birds in three stages of disease development when symptoms are first noted: clinically ill, incubating with no outward signs of illness, and unaffected susceptible. Therefore, in most instances, the entire flock is treated instead of just the clinically ill

Table 35.3. Recommended dosages of antimicrobial drugs for prophylactic and therapeutic use in poultry.

Drug	Prophylactic		Therapeutic		Other
	g/ton (US)	mg/L	g/ton (US)	mg/L	
Amoxicillin					15-20 mg/kg ^c
Apramycin					0.25-0.5 g/l ^c
Bacitracin	50-100		100-200		
Ceftiofur					0.08-0.2 mg/chick ^a
Clopidol	113-227				
Chlortetracycline	50-200		100-500	106-264.5	
Dimetridazole	98-197		453		
Doxycycline				50	
Enrofloxacin					10 mg/kg ^c
Erythromycin	92.5-185		92.5-185	115.6-250	
Gentamicin					0.2-1.0 mg/chick ^a
Lincomycin	2		2	17	
Lincomycin + spectinomycin				530-833	50-65 mg/lb
Neomycin		35-80	35-226		5 mg/lb
Nitarson	170				
Nifursol	50				
Novobiocin			200-350		7-14 mg/lb
Nystatin	50		50-100		
Ormetoprim + sulfadimethoxine	34.05 + 56.75 to 68.1 + 113.5		136.2 + 227	to 272.4 + 454	
Oxytetracycline	50-200		100-500	26.5-105.8	6.25-200 mg/bird
Oxytetracycline + neomycin			100-200	+ 35-40	
Penicillin	50-100		100		1,500,000 IU/gal
Penicillin + streptomycin					20,000 IU + 25 mg/lb
Ronidazole	54.4				
Sarafloxacin					0.1 mg/chick ^a
					20-40 ppm ^c
Spectinomycin		132		264-530	2.5-10 mg/chick ^a
Spiramycin				400	
Streptomycin				66-100	25 mg/lb
Sulfachlorpyridazine + trimethoprim					24 mg total activity/kg ^c
Sulfadiazine + trimethoprim					15 mg total activity/kg ^c or 300 g total activity/ metric ton (in feed)
Sulfadimethoxine				250-500	
Sulfamethazine				1000	110-273 mg/kg
Sulfaquinoxaline + trimethoprim					30 mg total activity/kg ^c
Sulfaquinoxaline				397	
Sulfathiazole				1000	
Tetracycline		100-200		200-400	25-50 mg/bird ^b
Tylosin			800-1000	530	15-25 mg/bird ^b
Virginiamycin	5-20				

Notes

^aSubcutaneous; ^bintranasal; ^coral.

While the above dosages are those recommended by the manufacturer, the approved dose, duration, and withdrawal period should always be confirmed prior to drug use in poultry for human consumption. Table based on AC Tanner, 3rd ed. this text.

birds. Such strategic medication in anticipation of major disease spread is justifiable under conditions of good husbandry practices. Finally, responsible therapy also allows sufficient withdrawal time for the antimicrobial to be eliminated from meat or eggs destined for human consumption.

Additional information on judicious antimicrobial use is available in Chapter 27 and from the American Veterinary Medical Association (<http://www.avma.org/scienact/jtua/default.asp>).

Bibliography

- Afifi NA, El-Sooud KA. 1997. Tissue concentrations and pharmacokinetics of florfenicol in broiler chickens. *Dtsch Tierarztl Wschr* 104:178.
- Barnes HJ, et al. 2003. Colibacillosis. In Saif YM, ed. *Diseases of Poultry*, 11th ed., 631-652. Ames, IA: Blackwell Publishing.
- Blom L. 1975. Residues of drugs in eggs after medication of laying hens for eight days. *Acta Vet Scand*. 16:396.
- Bradbury JM, et al. 1994. In vitro evaluation of various antimicrobials against *Mycoplasma gallisepticum* and *Mycoplasma synoviae* by the microbroth method and comparison with a commercially prepared test system. *Avian Pathol* 23:105.
- Charleston B, et al. 1998. Comparison of the efficacies of three fluoroquinolone antimicrobial agents given as continuous or pulsed water medication, against *Escherichia coli* infection in chickens. *Antimicrob Ag Chemother* 42:83.
- Clary BD, et al. 1981. The potentiation effect of citric acid on aureomycin in turkeys. *Poult Sci* 60:1209.
- Corrier DE, et al. 1999. Presence of *Salmonella* in the crop and ceca of broiler chickens before and after preslaughter feed withdrawal. *Poult Sci* 78:45.
- Glisson JR. 1997. Correct use of fluoroquinolones in the poultry industry. *Turkey World*. Mar-Apr pp. 24-26.
- Glisson JR. 1998. Bacterial respiratory diseases of poultry. *Poult Sci* 77:1139.
- Glisson JR, et al. 2004. Comparative efficacy of enrofloxacin, oxytetracycline, and sulfadimethoxine for the control of morbidity and mortality caused by *Escherichia coli* in broiler chickens. *Avian Dis* 48:658.
- Goren E, et al. 1984. Some pharmacokinetic aspects of four sulphonamides and trimethoprim, and their therapeutic efficacy in experimental *Escherichia coli* infection in poultry. *Vet Quart* 6:134.
- Gupta RC, Sud SC. 1978. Sulphaquinoxaline in the poultry. *Ind J Anim Res* 12:91.
- Hofacre CL, et al. 1998. Use of Aviguard, virginiamycin or bacitracin MD in experimental *Clostridium perfringens*-associated necrotizing enteritis. *J Appl Poult Res* 7:412.
- Kinney N, Robles A. 1994. The effect of mixing antibiotics with Marek's disease vaccine. *Proc 43rd Western Poultry Disease Conf*, 96-97.
- Laber G, Schutze E. 1977. Blood level studies in chickens, turkey poults, and swine with tiamulin, a new antibiotic. *J Antibiot*. 30:1112.
- McCapes RH, et al. 1976. Injecting antibiotics into turkey hatching eggs to eliminate *Mycoplasma meleagridis* infection. *Avian Dis* 19:506.
- Perelman B, et al. 1986. Clinical and pathological changes caused by the interaction of lasolocid and chloramphenicol in broiler chickens. *Avian Pathol* 15:279.
- Righter H F, et al. 1970. Tissue-residue depletion of sulfaquinoxaline in poultry. *Am J Vet Res*. 31:1051.
- Righter HE, et al. 1973. Tissue residue depletion of sulfaquinoxaline in turkey poults. *J Agr Food Chem* 21:412.
- Russell SM. 2003. The effect of airsacculitis on bird weights, uniformity, fecal contamination, processing errors and population of *Campylobacter* spp. and *Escherichia coli*. *Poult Sci* 82:1326.
- Stanley WA, et al. 2001. Case Report—Monitoring *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infection in an experimental line of broiler chickens after treatment with enrofloxacin. *Avian Dis* 45:534.
- Sumano LH, et al. 2004. Influence of hard water on the bioavailability of enrofloxacin in broilers. *Poult Sci* 83:726.
- Vernimb GD, et al. 1977. Effect of gentamicin and early mortality and later performance of broiler and leghorn chickens. *Avian Dis* 20:706.
- Zamon Q, et al. 1995. Experimental furazolidone toxicosis in broiler chicks: effect of dosage, duration and age upon clinical signs and some blood parameters. *Acta Vet Hung* 43:359.
- Zhao S, et al. 2005. Antimicrobial susceptibility and molecular characterization of avian pathogenic *Escherichia coli* isolates. *Vet Microbiol* 107:215.

Antimicrobial Drug Use in Companion Birds

Keven Flammer

The companion birds include members of the orders Psittaciformes (e.g., parakeets, parrots, lorries, cockatoos and macaws), Passeriformes (e.g., canaries and finches) and Columbiformes (e.g., pigeons and doves). Psittacine birds are the most common pet birds in the US; over 50 species are commonly seen in veterinary practice. Microbial diseases are common and use of antimicrobial drugs is an important part of avian practice. Optimal treatment regimens can be developed if the principles of rational antimicrobial therapy are integrated with the unique behavioral and physiological characteristics of birds.

The general approach to selecting an avian antimicrobial treatment regimen is similar to other species. The site and cause of infection should be identified and the minimal inhibitory concentrations (MIC) of potentially effective antimicrobial drugs determined. Selection of the most appropriate drug will then depend on the severity of illness, site of infection, pharmacokinetic and pharmacodynamic properties of the selected drugs, and the routes of administration that can be accomplished by the owner or veterinary staff. Additional considerations are drug side effects, toxicity and cost.

Establishing the Cause and Site of Infection

A wide variety of primary and secondary bacterial pathogens have been identified in companion birds (Table 36.1); however, some are more common than others. In psittacine birds, Gram-negative bacterial infections are most common, especially those caused by *Escherichia coli*, *Klebsiella* spp., and *Pseudomonas aeruginosa*. Other Gram-negative bacteria include

Bordetella, *Salmonella*, *Proteus*, *Serratia*, *Yersinia* and *Pasteurella* species. Gram-positive bacterial pathogens include *Staphylococcus aureus* and *Enterococcus*. *Chlamydia psittaci* is the most important intracellular pathogen; *Mycobacterium avium* and *M. genavense* are occasionally seen. Anaerobes are relatively uncommon, although clostridial infections of the alimentary tract do occur. Similar pathogens are found in canaries and pigeons; *Enterococcus fecalis* is an important cause of respiratory disease in canaries and there is a high incidence of *Salmonella* and *Streptococcus bovis* in pigeons.

Mycotic infections are also important (Table 36.1). Yeasts most commonly affect the alimentary tract and common pathogens include *Candida albicans* and avian gastric yeast. Hyphal fungi are important pathogens of the respiratory tract and, occasionally, the eye and skin. *Aspergillus fumigatus* and *A. niger* are the most common isolates; *Scedosporium*, *Mucor*, *Rhizopus*, *Penicillium*, and other opportunists may rarely infect immunocompromised birds.

In companion birds, septicemia and infections of the alimentary tract, respiratory tract, and liver are the most common sites of microbial infection. It is important to note that simply culturing a potential pathogen is not an indication for antimicrobial drug treatment. It is not unusual to culture small numbers of Gram-negative bacteria or yeast from the cloaca and choana of apparently healthy birds. Treatment may be indicated if the organism is present in large numbers and there are accompanying clinical signs. Physical exam findings, results of clinical laboratory tests, and a Gram-stain of material from the suspected site of infection can help determine if a microbial infection is the cause of illness.

Choosing an Antimicrobial Regimen

To be effective, the pathogen must be susceptible to the drug at concentrations that are achievable in birds. Some microbial agents have known susceptibility (e.g., *Chlamydophila psittaci* is sensitive to doxycycline), but most will require a susceptibility test to determine the most effective drugs. Susceptibility tests reporting minimal inhibitory concentrations (MIC) are quantitative and provide the most useful information to guide drug selection. Disk diffusion tests can be used, but it is important to recognize that the designations of susceptible, intermediate, and resistant may not correlate with treatment success in birds. These designations are based on the achievable drug concentrations in humans (or in some cases, dogs) and it may be difficult to achieve similar concentrations in birds. See Chapter 2 for additional information on susceptibility testing.

Companion birds often hide signs of disease and may present at an advanced stage of illness. If a bacterial infection is strongly suspected, it may be necessary to start empirical treatment before the results of culture and susceptibility tests are available. Table 36.1 provides a list of diseases and suggested choices for initiating antimicrobial therapy. In companion birds, Gram-negative bacterial infections are most common, especially those caused by *Escherichia coli*, *Klebsiella* spp., and *Pseudomonas aeruginosa*. Chlamydiosis most commonly occurs in birds recently obtained from commercial sources (e.g., pet stores, flea markets and breeders). *Salmonella* is common in pigeons. If these organisms are suspected, a broad-spectrum antibiotic with excellent Gram-negative spectrum is most appropriate for initiating empirical treatment; doxycycline is preferred if chlamydiosis is likely. Susceptibility data are sparse; however, one study of the MIC₉₀ values for Gram-negative bacteria isolated from psittacine birds suggests that resistance to many first generation antimicrobials (e.g., penicillin, ampicillin, cephalexin, chloramphenicol and tetracycline) may be common in psittacines (Table 36.2). Because of potential resistance, avian veterinarians often use fluoroquinolones and advanced generation beta-lactams for initial treatment in severely ill birds. The treatment plan can be modified once the bird is stable and results of laboratory testing are available.

The frequency and route of administration are important considerations when choosing a dosage regi-

men. Most birds will need to be captured and restrained to deliver medication, so treatment regimens with a longer dosage interval are preferred. In sick birds, a parenteral route of administration should be used to rapidly establish effective drug concentrations. Once a bird is clinically stable, it may be relinquished to the owner's care to complete antimicrobial therapy. Birds can be difficult to medicate and the procedure is often stressful for both the bird and bird owner. If oral medication is used, low-volume, palatable drug formulations can aid treatment success. Some avian veterinarians favor use of IM injection because bird restraint and drug delivery may be easier with this route. Additional pros and cons of different routes of administration are listed below. Regardless of the treatment regimen, it is useful to check compliance and offer assistance after a few days of treatment.

Choosing the dose can be challenging because drug formularies often list a wide range of recommended dosages. This is partly because there is sparse data on the pharmacokinetics of antibiotics in many species of psittacine birds. Many dosage regimens are empirically derived or extrapolated from other species. Table 36.3 provides suggested doses for selected commonly used antimicrobial drugs. However, even doses based on pharmacokinetic studies often represent only a single species. Fortunately, research on antimicrobial drug use in companion birds is progressing and dosing recommendations are updated frequently.

Basic pharmacological principles should be considered when evaluating which dose to use. Drugs showing time-dependent efficacy (e.g., beta-lactams, macrolides, tetracyclines and trimethoprim-sulfonamides) must be dosed frequently enough to maintain plasma concentrations above the target MIC for most of the dosing interval. Birds rapidly excrete most beta-lactam drugs, so penicillins and cephalosporins should be dosed at least 3–4 times daily unless pharmacokinetic data demonstrates less frequent administration is adequate [for example, some Gram-positive bacteria may require only q12h administration (Dorrestein, 1986)]. Concentration-dependent antibiotics (e.g., fluoroquinolones and aminoglycosides) can probably be dosed once daily if high peak concentrations are achieved. Since peak concentration may depend on the route of administration, parenteral routes may be required to achieve the desired concentration for resistant organisms.

Controlled studies involving large numbers of dif-

Table 36.1. Antimicrobial drug selection in companion avian infections.

Site or Type of Infection	Diagnosis	Common Organisms	Suggested Drugs	Comments
Severe illness, cause unknown	Septicemia, multiple organ infection	Aerobic bacteria, especially <i>E. coli</i> and <i>Klebsiella</i>	Enrofloxacin; piperacillin; cefotaxime	Use IV, IM, or SQ route.
		<i>Pseudomonas aeruginosa</i>	Amikacin + piperacillin or cefotaxime; meropenem	Maintain hydration to avoid aminoglycoside toxicity.
		<i>Chlamydophila psittaci</i>	Doxycycline	Use IV route if severely ill, oral or IM if stable.
Mild illness, cause unknown	Septicemia, multiple organ infection	<i>Aspergillus</i>	Amphotericin B	Use IV route.
		Aerobic bacteria, especially <i>E. coli</i> and <i>Klebsiella</i>	Enrofloxacin; trimethoprim sulfamethoxazole; ampicillin/clavulanic acid	
		<i>Pseudomonas aeruginosa</i>	Amikacin + piperacillin; cefotaxime; meropenem	Maintain hydration to avoid aminoglycoside toxicity.
Respiratory	Rhinitis/sinusitis	<i>Chlamydophila psittaci</i>	Doxycycline	Oral administration via medicated food or water.
		<i>Aspergillus</i>	Itraconazole	
		Aerobic bacteria, especially <i>E. coli</i> and <i>Klebsiella</i>	Enrofloxacin; piperacillin; cefotaxime	Gently lavage nares/sinus with saline to remove debris. Treat for at least one week after signs resolve. Chronic cases may require surgical debridement to remove nidus of infection.
		<i>Pseudomonas aeruginosa</i>	Amikacin + piperacillin; cefotaxime; ciprofloxacin; meropenem; enrofloxacin if MIC <0.5 µg/ml	Maintain hydration to avoid aminoglycoside toxicity.
		<i>Candida albicans</i>	Fluconazole	
	Pneumonitis/airsacculitis	<i>Aspergillus</i>	Amphotericin B	Nebulize, nasal flush.
			Itraconazole	Monitor toxicity.
		<i>Mycoplasma</i>	Enrofloxacin; doxycycline	Role in psittacine sinusitis uncertain.
		<i>Chlamydophila psittaci</i>	Doxycycline	
		<i>Aspergillus</i> ; opportunistic fungi	Amphotericin B + itraconazole and/or terbinafine	Amphotericin B: Nebulize 2-3x daily; IV for 3-5 days if bird is severely debilitated.
Gastrointestinal	Oral, gastric, intestinal candidiasis			Itraconazole: Oral administration only. Monitor potential toxicity, especially in African grey parrots.
				Terbinafine: Combine with or substitute for itraconazole. Treat for at least one month after resolution of clinical signs.
				Use IV, IM or SQ route.
		Aerobic bacteria, especially <i>E. coli</i> and <i>Klebsiella</i>	Enrofloxacin; piperacillin; cefotaxime	
	Bacterial enteritis	<i>Pseudomonas aeruginosa</i>	Amikacin + piperacillin; cefotaxime; ciprofloxacin; enrofloxacin if MIC <0.5 µg/ml; meropenem.	Base treatment on MIC. Maintain hydration to avoid toxicity when using aminoglycosides.
		<i>Chlamydophila psittaci</i>	Doxycycline	
		<i>Scedosporium</i>	Itraconazole + terbinafine	Rarely reported.
		<i>Candida</i>	Fluconazole	Can treat oral lesions with topical Amphotericin B.
	Bacterial enteritis	Opportunistic aerobic bacteria, especially <i>E. coli</i> and <i>Klebsiella</i>	Nystatin	Use oral route. Treat for 5-7 days.
		<i>Campylobacter</i>	Enrofloxacin; other fluoroquinolones; trimethoprim-sulfa	
			Doxycycline	Rare in psittacines. Occasionally seen in finches.

(continued)

Table 36.1. Antimicrobial drug selection in companion avian infections. (continued)

Site or Type of Infection	Diagnosis	Common Organisms	Suggested Drugs	Comments
		Spore-forming bacteria (probably <i>Clostridia</i>)	Clindamycin; metronidazole	Common cause of odiferous droppings. <i>C. perfringens</i> may cause acute mortality.
	Avian gastric yeast	<i>Macrorhabdus ornithogaster</i>	Amphotericin B	Give orally. Impossible to clear infection in all affected birds.
	Bacterial enteritis	<i>Pseudomonas aeruginosa</i>	Gentamicin PO if showing mild signs. If ill, use amikacin + piperacillin or cefotaxime; ciprofloxacin; enrofloxacin if MIC <0.5 µg/ml; meropenem.	Check husbandry for environmental sources (esp. water sources, contaminated food, etc.)
	Cloacitis	Opportunistic aerobic and anaerobic bacteria	Enrofloxacin or beta-lactam + clindamycin or metronidazole. Topical silver sulfadiazine cream.	Most common in cockatoos. Associated septicemia may cause severe debilitation.
Nervous	Pharyngitis	Spiral bacteria	Doxycycline	Reported in cockatiels.
	Bacterial meningitis/encephalitis	Opportunistic aerobic pathogens	Cefotaxime; doxycycline; enrofloxacin	Rare. Treat aggressively. Use high end of the dosage range. Prognosis poor.
	Mycoplasma encephalitis	<i>Mycoplasma</i>	Doxycycline; enrofloxacin	Rare; prognosis poor.
Ophthalmic	Bacterial keratitis, mild ulceration	Opportunistic bacteria	Topical bacitracin-neomycin-polymyxin B combination	Topical ciprofloxacin gentamicin, and topical tetracycline are other options.
	Bacterial keratitis, severe	<i>Pseudomonas aeruginosa</i>	Topical tobramycin; topical amikacin	
	Fungal keratitis	<i>Aspergillus</i> and other opportunistic fungi	Topical miconazole; natamycin	Oral itraconazole
	Manifestation of systemic disease	<i>Chlamydomydia psittaci</i>	Topical tetracycline	Also treat with oral doxycycline.
Skin	Dermatitis	Opportunistic aerobic bacteria	Enrofloxacin; trimethoprim sulfamethoxazole; beta-lactams. Topical silver sulfadiazine cream.	Must also treat underlying cause. Often find multiple classes of organisms.
	<i>Staph. dermatitis</i>	<i>Staphylococcus aureus</i>	Cephalothin; oxacillin; trimethoprim sulfonamide.	Resistant <i>Staphylococcus</i> uncommon in birds. MRSA occasionally seen.
		Opportunistic yeast	Fluconazole; topical amphotericin B cream.	
		Opportunistic hyphal fungi	Itraconazole; topical amphotericin B cream.	
Reproductive	Salpingitis (oviduct)	Opportunistic aerobic bacteria, especially <i>E. coli</i> and <i>Klebsiella</i>	Fluoroquinolones; beta-lactams	Check for a retained or ruptured egg. Resolution may require surgery.
Peritonitis		Mixed bacterial opportunists, especially Gram-negative bacteria	Enrofloxacin; piperacillin; cefotaxime	Consider egg yolk peritonitis if bird is female.
Multiple organs	Mycobacteriosis	<i>Mycobacterium avium</i> <i>Mycobacterium genavense</i>	Long-term multiple drug therapy.	<i>M. genavense</i> may be zoonotic. Treatment is complex.
Otitis media		Gram-negative bacteria, <i>E. coli</i> and <i>Klebsiella</i>	Fluoroquinolones, beta-lactams	Most commonly reported in nestling macaws. Lavage ear and treat with topical amikacin.
		<i>Pseudomonas aeruginosa</i>	Amikacin + piperacillin; cefotaxime; ciprofloxacin; enrofloxacin if MIC <0.5 µg/ml; meropenem	Difficult to deliver multiple injections to juvenile birds. Use oral route if an effective drug is available.

Table 36.2. Minimal inhibitory concentration for 90% (MIC₉₀, µg/ml) of Gram-negative bacteria isolated from the cloaca of psittacine birds.^a

Drug	<i>E. coli</i>	# ^b	<i>Klebsiella</i> spp.	#	<i>Pseudomonas</i> spp.	#	<i>Proteus</i> spp.	#	<i>Enterobacter</i> spp.	#	<i>Salmonella</i> <i>typhimurium</i>	#
Penicillins												
Ampicillin	>16	391	>16	50	>16	27	>16	18	>16	69	>16	2
Amoxicillin + clavulanic acid	8	—	8	38	>16	15	>16	11	>16	54	—	—
Carbenicillin	>16	145	>512	16	>512	27	32	18	>256	16	—	—
Ticarcillin	>16	249	>64	36	>64	15	>64	11	32	54	<8	2
Cephalosporins												
Cefazolin	>16	218	>16	35	>16	12	>16	7	>16	38	4	2
Cephalothin	16	392	>32	54	>32	20	>32	18	>32	71	4	2
Cefoxitin	16	390	8	52	>32	24	>32	18	>32	70	<2	2
Cefotaxime	<8	362	<8	50	>32	24	4	14	16	55	<8	2
Ceftriaxone	<0.5	240	16	33	64	14	>64	10	1	42	<0.5	2
Fluoroquinolones												
Ciprofloxacin	<0.03	200	0.25	23	0.12	13	<0.03	11	0.25	37	—	—
Norfloxacin	<1	250	<1	38	4	15	<1	11	<1	51	<1	2
Other												
Erythromycin	32	389	64	54	>64	25	>64	18	>64	72	<16	2
Clindamycin	>8	16	—	—	—	—	>8	4	>8	4	—	—
Trimethoprim + sulfamethoxazole	<1/19	333	4/76	54	>4/76	26	<1/19	18	<4/76	73	<1/19	2
Tetracycline	>16	393	>16	54	>16	26	>16	18	>16	73	>16	2
Chloramphenicol	>16	393	>16	51	>16	26	16	16	>16	73	4	2
Imipenem	0.5	249	2	38	>8	15	8	11	2	53	<0.05	2
Moxalactam	<4	143	<4	16	>64	10	>64	7	<4	18	—	—
Nitrofurantoin	32	389	64	54	>64	25	>64	18	>64	72	<16	2

^aData from clinical laboratories, North Carolina State University 1985–1990.^bNumber of isolates tested.

Adapted from Flammer (1992).

ferent avian species are lacking, so veterinarians should monitor treatment efficacy and potential toxicity. This is especially important when using drugs with a narrow therapeutic range or treating an unfamiliar species. Other chapters in this book should be consulted to learn more about specific antimicrobial drugs and their potential side effects and contraindications.

Using broad-spectrum antimicrobials may impact normal gut flora. Psittacine birds have predominately Gram-positive gut flora, and reduction of this flora after treatment can render the birds more susceptible to secondary infections by yeast and Gram-negative bacterial opportunists. This is especially common when treating nestling birds or when using prolonged antimicrobial therapy in adults [e.g., treatment for chlamydiosis (Flammer, 1994)]. The incidence of secondary infections can be reduced by maximizing husbandry during treatment. In addition, birds that have

sustained long-term treatment should be cultured to identify potential opportunistic superinfections.

Anatomical and Physiological Considerations

Differences in anatomy and physiology may alter drug pharmacology in birds as compared to mammals. For example, granuloma formation is a common avian response to infection by many microbial agents. Granuloma formation can inhibit drug penetration so surgical debridement, use of lipophilic drugs, and prolonged treatment may be needed to improve the success of treatment.

In mammals, gastric emptying and drug dissolution are often the rate limiting steps for oral drug absorption. Companion birds have a crop, and passage of ingesta from the crop may delay oral drug absorption. For example, a lag phase of 20–40 minutes was ob-

Table 36.3. Conventional dosage regimens for antimicrobial drugs in companion birds.^a

Drugs	Dose (mg/kg)	Interval (hr)	Route	Study ^b / Species ^c	Ref ^d	Comments
Penicillins						
Ampicillin sodium	150	12-24	IM	Pk / pigeons	1	Gram-positives only.
Ampicillin trihydrate	25	12-25	PO	Pk / pigeons	1	Gram-positives only.
	125-175	12-25	PO	Pk / pigeons	1	
	100	4	IM	Pk / Amazon parrots	2	
	150-200	8-12	PO	Pk / Amazon parrots	2	
Amoxicillin sodium	50	12-24	IM	Pk / pigeons	1	Gram-positives only.
	250	12-24	IM	Pk / pigeons	1	
Amoxicillin trihydrate	20	12-24	PO	Pk / pigeons	1	Gram-positives only.
	100	12-24	PO	Pk / pigeons	1	
	150-175	4-8	PO	Empirical / psittacines		
Amoxicillin + clavulanic acid	50/10	8-12	IM	Pk / collared doves	3	Gram-positives only.
	100/25	8-12	PO	Pk / collared doves	3	
	60-120	8-12	IM	Pk / collared doves	3	
	125-250	8	PO	Pk / collared doves	3	
	125	8	PO	Pk / blue fronted Amazon parrots	4	
Piperacillin	75-100	4-8	IM	Pk / blue-fronted Amazon parrots	5	
	200	6-8	IM	Empirical / psittacines		
Ticarcillin	200	2-4	IM	Pk / blue-fronted Amazon parrots	6	
Cephalosporins						
Cephalothin	100	6	IM	Pk / pigeon	7	
Cephalexin	35-50	6	PO	Pk / pigeon	7	
Ceftiofur	10	4	IM	Pk / cockatiels	8	
	10	8	IM	Pk / orange-winged Amazon parrots	8	
Cefotaxime	75-100	4-8	IM	Pk / blue-fronted Amazon parrots	5	
Ceftazidime	50-100	4-8	IM	Empirical		
Ceftriaxone	75-100	4-8	IM	Pk / blue-fronted Amazon parrots	5	
Aminoglycosides						
Amikacin	15-40	24	IM, IV	Pk / cockatiels, blue-fronted Amazon parrots, African grey parrots	9 6 10	Preferred aminoglycoside. Potentially nephrotoxic.
Gentamicin	2.5-10	24	IM	Pk / cockatiels, scarlet macaws, rose breasted cockatoos	9 11	Nephrotoxic.
Tobramycin	2.5-10	24	IM	Empirical		Empirical; based on gentamicin studies. Used for <i>Pseudomonas aeruginosa</i> .
Fluoroquinolones						
Enrofloxacin	7.5-15	12-24	IM	Pk / African grey parrots	12	IM injection causes muscle irritation.
	7.5-15	12-24	SC	Pk / African grey parrots	13	Inject into subcutaneous fluid pocket containing lactated Ringer's solution. Double the dose when using q24h administration.
	15-30	24	SC, PO	Pk / African grey parrots	13	
	200 mg/L	24	Water	Plasma concentration / parrots	14	Achieves low plasma concentrations in psittacines.
Marbofloxacin	2.5-5	24	PO	Pk / blue and gold macaw	15	
Tetracyclines						
Oxytetracycline, long-acting (LA 2004r™, Pfizer)	50-100	48-72	IM, SC	Pk / Goffin's cockatoo	16	<i>Chlamydophila psittaci</i> . Causes irritation at the site of injection.
Doxycycline	25	12	PO	Pk / pigeon	1	<i>Chlamydophila psittaci</i> . Dose in birds with access to grit.
	7.5	12	PO	Pk / pigeon	1	<i>Chlamydophila psittaci</i> . Dose in birds with no access to grit.

Table 36.3. Conventional dosage regimens for antimicrobial drugs in companion birds.^a

Drugs	Dose (mg/kg)	Interval (hr)	Route	Study ^b / Species ^c	Ref ^d	Comments
	25-35	24	PO	Psittacines / empirical		<i>Chlamydophila psittaci</i>
	300 mg/kg food	24	Food	Plasma concentration / budgerigars	17	<i>Chlamydophila psittaci</i> . Diet = 1:4 mixture of hulled oat groats and hulled millet. Coat seed with sunflower oil (~6 ml/kg seed).
	300-500 mg/kg food	24	Food	Plasma concentration / cockatiels	18	Diet = 60:40 mixture of hulled millet and hulled sunflower seeds. Coat seed with sunflower oil (~6 ml/kg seed).
	300 mg/L	24	Water	Plasma concentration / cockatiels	18	
	400-800 mg/L	24	Water	Plasma concentration / orange-winged Amazon parrot, African grey parrot, Goffin's cockatoo	19	
Doxycycline injectable (Vibrovenos®, Pfizer)	75-100	5-7 days	IM	Pk / pigeons Pk / psittacines	1 20	Use lower doses in macaws and cockatoos.
Trimethoprim and sulfonamides						
Trimethoprim	15-20	8	PO	Pk / pigeons	1	
Trimethoprim-sulfamethoxazole	10/50	12	PO	Pk / pigeons	1	
Trimethoprim-sulfatroxazole	10/50	24	PO	Pk / pigeons	1	
Trimethoprim-sulfamethoxazole	20/100	12	PO	Empirical		May cause regurgitation, especially in macaws.
Other						
Metronidazole	20-50	12	PO	Empirical		Anaerobes
Tylosin	25	6	IM	Pk / pigeons	21	
Clindamycin	25-50	8-12	PO	Empirical		Gram-positives and anaerobes
Antifungals						
Amphotericin B	1.5	8	IV	Empirical		<i>Aspergillus</i> and hyphal fungi
	1.0	8-12	IT	Empirical		<i>Aspergillus</i> and hyphal fungi
	1.0 mg/ml	8-12	Neb	Empirical		<i>Aspergillus</i> and hyphal fungi
	100 mg/kg	12	PO	Empirical		Avian gastric yeast
Ketoconazole	20-30	12	PO	Pk / Amazon parrots and cockatoos	22	Yeast, +/- <i>aspergillus</i>
Fluconazole	10-20	24	PO	Pk / African grey parrots, blue-fronted Amazon parrots, Goffin's cockatoos	23	Yeast. Higher dose may be toxic in African grey parrots.
Itraconazole	5-10	24	PO	Pk / blue-fronted Amazon parrot	24	<i>Aspergillus</i> and hyphal fungi
	6	12	PO	Pk / pigeon	25	<i>Aspergillus</i> and hyphal fungi
	2.5 - 5	24	PO	Empirical / African grey parrot		Itraconazole may be toxic in some African grey parrots, even at the low dose indicated here.
Nystatin	200,000-300,000 IU/kg	8-12	PO	Empirical		Yeast. Not absorbed from the GI tract. Must come in contact with the yeast.

^aAdapted from Dorrestein (2000).

^bPk indicates that the recommendation is based on pharmacokinetic studies in the listed species. Empirical indicates that the recommendation is based on anecdotal reports (no published kinetic data available for pigeons or psittacine birds).

^cScientific names: pigeon (*Columba livia*), blue fronted Amazon (*Amazona aestiva*), cockatiel (*Nymphicus hollandicus*), collared dove (*Streptopelia decaocto*), orange winged Amazon (*Amazona amazonica*), African grey parrot (*Psittacus erithacus erithacus*), scarlet macaw (*Ara macao*), rose breasted cockatoo (*Cacatua roseicapilla*), blue and gold macaw (*Ara ararauna*), Goffin's cockatoo (*Cacatua goffini*), budgerigar (*Melopsittacus undulatus*).

^dReferences: 1, Dorrestein 1986; 2, Ensley 1981; 3, Dorrestein et. al. 1998; 4, Orosz et. al. 2000; 5, Flammer 1990; 6, Schroeder et. al. 2001; 7, Bush et. al. 1981; 8, Tell et. al. 1998; 9, Ramsay et. al. 1993; 10, Gronwall et. al. 1989; 11, Flammer and Clark et. al. 1990; 12, Flammer et. al. 1991; 13, Flammer 2005; 14, Flammer et. al. 2002; 15, Carpenter, et. al. 2003; 16, Flammer and Aucoin, et. al. 1990; 17, Flammer et. al. 2003; 18, Powers et. al. 2000; 19, Flammer et. al. 2001; 20, Jakoby and Gylstorff 1983; 21, Bush et. al. 1982; 22, Kollias et. al. 1986; 23, Flammer K 1996; 24, Orosz et. al. 1996; 25, Lumeij et. al. 1995.

served in studies investigating the pharmacology of oral suspensions of doxycycline in fasted birds (Flammer, unpublished observation, 2005). There is little absorption from the crop, and its neutral pH may precipitate some drugs that are solubilized in acid or base (e.g., chlortetracycline), further delaying absorption (Dorrestein, 1986).

Alimentary tract motility in birds also differs from mammals (Denbo, 2000). Birds have a two-part stomach composed of the proventriculus and ventriculus. Grit is retained in the ventriculus and may expose orally administered drugs to high concentrations of calcium and magnesium. This can reduce the absorption of tetracyclines and fluoroquinolones (Dorrestein, 1986). There is also both normograde and retrograde movement of ingesta through the proventriculus, ventriculus, and small intestine. This might expose acid-sensitive drugs to greater degradation by gastric acids. Companion birds also have a short intestinal tract that may limit drug absorption, especially when food is present and competes for absorption.

The lower respiratory system of birds consists of the lungs and air sacs (Powell, 2000). The air sacs are poorly vascularized and topical drug delivery via nebulization may be needed to augment systemic drug administration. At rest, birds may ventilate only a small portion of their total air sac volume, so nebulization may be enhanced by gently stimulating the bird to increase respiration and promote greater drug penetration.

The renal system of birds differs considerably from mammals (Goldstein, 2000; Fraizer et al., 1995). Avian kidneys contain both mammalian and reptilian nephrons and may excrete drugs differently than expected from mammalian physiology. Uric acid is the major end product of avian nitrogen metabolism and is produced in the liver. Sulphonamide drugs may be excreted via some of the same metabolic pathways as uric acid, so caution should be used if sulfa drugs are given to uricemic birds (Quesenberry, 1988). Birds lack a bladder, and waste from the kidney is transported directly to the cloaca. Cloacal contents can be refluxed into the colon to promote additional water absorption. As a consequence, avian water balance may be independent of the glomerular filtration rate and renally excreted drugs may face reabsorption in the colon. As a final consideration, birds have a renal portal system. Theoretically, renally excreted drugs could face a first-pass effect before reaching systemic circulation if injected into the leg muscles.

Routes of Administration

The route of administration will depend on the drug, available drug formulation, condition of the bird, and ability of the owner and/or veterinary staff to deliver the drug. Severely ill birds should be treated using parenteral routes to quickly establish effective drug concentrations. Achievable plasma concentrations are often route-dependent. As a guideline, concentrations follow the following pattern: IV > IM \geq SC > PO > medicated food or water (Flammer, 1994).

Intravenous (IV) Route: It is difficult to deliver IV drugs in birds so this method is usually reserved for one-time administration of antimicrobials or emergency drugs. Birds can be catheterized, but it is more difficult to maintain IV catheters in birds than in other small animals. The right jugular and right and left brachial veins are the most accessible in psittacines. The medial metatarsal vein is accessible in pigeons.

Intraosseous (IO) route: Fluids given via the intraosseous route quickly reach systemic circulation (Aguilar et al., 1993). Intraosseous catheters can be installed in the distal ulna or tibiotarsus. This route is most often used to administer fluids; however, it is an acceptable route for IV antimicrobial drug formulations. Care should be taken to flush fluid through the IO catheter and bone to avoid leaving concentrated drug in the IO site.

Intramuscular (IM) Route: The pectoral muscles are the most accessible sites for IM administration in parrots and passerines; the leg muscles are sometimes used in racing pigeons. Small needle size (25–30 gauge) and small volume of injection are necessary. The author prefers to use injection volumes that are less than 1 ml/kg. Irritating drugs (e.g., enrofloxacin and tetracyclines) should be avoided unless there is a compelling reason to use this route.

Subcutaneous (SC) route: Medications can be given subcutaneously in the groin, axilla and the dorsal region between the shoulders. Non-irritating drugs are preferred. Injectable tetracyclines (e.g., oxytetracycline) have been used, but can cause skin sloughs (Flammer et al., 1990). Enrofloxacin can be injected into a SC pocket of lactated Ringer's solution and achieve plasma concentrations comparable to IM injection, without causing severe irritation (Flammer, 2005).

Oral (PO) route: Liquid solutions and suspensions are often used. Capsules can be given to pigeons but are difficult to administer to parrots and passerines.

(Dorrestein, 1984). Drugs that are unpalatable or require large volumes are more difficult to administer. Only non-irritating drugs should be used, as birds may aspirate drug into the trachea or pass it rostrally into the choanal slit. It can be surprisingly difficult to medicate psittacines via the oral route, so owner compliance should be verified if this route is chosen. As an alternative, drugs can be administered via a crop tube; however, this method is technically difficult and is usually performed in a veterinary hospital setting.

Medicated food: Medications can be added to palatable food vehicles such as mash diets and treat foods. It is difficult to monitor food (and therefore drug) consumption, so this route should be reserved for treatment of clinically stable birds with proven dosage regimens. Lower plasma drug concentrations are usually achieved than with other routes, so this method is used only to treat highly susceptible bacteria. It is important to use the same diet as is used in published methods, since food consumption is largely based on the energy content of the diet (Flammer, 1994).

Medicated water: Delivering medication via this route usually establishes low plasma drug concentrations. This route should be avoided unless there is data proving therapeutic plasma drug concentrations can be achieved. For example, water medicated with enrofloxacin at 200 mg/L achieves low, sustained plasma concentrations of 0.05–0.2 µg/ml (Flammer et al., 2002). Doxycycline medicated water has been shown to achieve plasma drug concentrations effective for treating chlamydiosis (~1 µg/ml) in cockatiels treated with 300 mg/L (Powers et al., 2000) and cockatoos and grey parrots treated with 400–800 mg/L (Flammer et al., 2001).

Topical: Topical drugs can be applied to the skin or eye. A minimal amount of topical cream or ointment should be used, as birds may ingest or spread medications into their feathers when preening. Where possible, water soluble formulations are preferred, as they are easier to wash off if the bird spreads them into the feathers. Silver sulfadiazine cream is a popular choice for treating avian skin infections because it has broad-spectrum activity and is easy to clean up.

Antimicrobials are occasionally injected directly into the site of infection. Intratracheal injection can be used to deliver topical amphotericin B (~1 ml/kg) to treat fungal infections of the trachea. Amphotericin B and clotrimazole have been used to topically treat fungal lesions on the air sacs. Topical antibiotics are some-

times used to treat upper respiratory infections via injection into the nares (nasal flush) or periorbital sinus (sinus flush).

Nebulization: Nebulization can be used to deliver topical medication to portions of the air sacs and lungs. It is most often used when treating respiratory fungal infections. A nebulizer that produces particles less than 3 microns in diameter should be used. Birds ventilate only a small portion of their respiratory tract at rest, so stimulation or mild exercise during nebulization might increase drug penetration. In studies investigating tylosin and oxytetracycline, nebulization achieved therapeutic local concentrations for approximately 4–6 hours, but did not establish therapeutic plasma concentrations (Locke et al., 1984; Dyer et al., 1987).

Bibliography

- Aguilar RF, et al. 1993. Osseous venous and central circulatory transit times of technetium-99m pertechnetate in anesthetized raptors following intraosseous administration. *J Zoo Wildlife Med* 24:488.
- Bush M, et al. 1981. Pharmacokinetics of cephalothin and cephalexin in selected avian species. *Am J Vet Res* 43:1014.
- Bush M, et al. 1982. Pharmacokinetics and tissue concentrations of tylosin in selected avian species. *Am J Vet Res* 42:1807.
- Locke D, et al. 1984. Tylosin aerosol therapy in quail and pigeons. *J Zoo An Med* 15:67.
- Carpenter JW, et al. 2003. Pharmacokinetics of marbofloxacin in the blue and gold macaw. *Proc Ann Conf Am Assoc Zoo Vet*, 79.
- Carpenter JW. 2005. *Exotic Animal Formulary*. St. Louis, MO: Elsevier Saunders.
- Dorrestein GM. 1986. Studies on the pharmacokinetics of some antibacterial agents in homing pigeons (*Columba livia*). Thesis, Utrecht University.
- Dorrestein GM, et al. 1987. Comparative study of ampicillin and amoxicillin after intravenous, intramuscular and oral administration in homing pigeons (*Columba livia*). *Res Vet Sci* 42:343.
- Dorrestein GM, et al. 1988. Pharmacokinetics of Baytril in homing pigeons after different administration routes. *Proc 6th Conf Avian Dis Munich*, 132.
- Dorrestein GM, et al. 1998. Comparative study of Synulox® and Augmentin® after intravenous, intramuscular and oral administration in collared doves (*Streptopelia decaocto*). *Proc 11th Symp Avian Dis Munich*, 42.
- Dorrestein GM. 2000. Antimicrobial drug use in companion birds. In: Prescott JF, Baggot JD, Walker RD, eds., *Antimicrobial Therapy in Veterinary Medicine*, 3rd edition. Ames, IA: Blackwell Publishing, 617.

- Denbo M. 2000. Gastrointestinal anatomy and physiology. In: Whittow GC, ed., *Sturkie's Avian Physiology*, 5th edition. San Diego, CA: Academic Press, 299.
- Dyer DC, et al. 1987. Antibiotic aerosolization: tissue and plasma oxytetracycline concentrations in parakeets. *Avian Dis* 31:677.
- Ensely PK, et al. 1981. A preliminary study comparing the pharmacokinetics of ampicillin given orally and intramuscularly to psittacines: Amazon parrots (*Amazona* spp.) and blue-naped parrots (*Tanygnathus lucionensis*). *J Zoo Anim Med* 12:42.
- Filippich LJ, et al. 1993. Drug trials against megabacteria in budgerigars. *Aust Vet Pract* 23:184.
- Flammer K. 1990. An update on psittacine antimicrobial pharmacokinetics. *Proc Ann Conf Assoc Avian Vet*, 218.
- Flammer K, Clark GH, et al. 1990. Adverse effects of gentamicin in scarlet macaws and galahs. *Am J Vet Res* 51:404.
- Flammer K, Aucoin DP, et al. 1990. Potential use of long-acting injectable oxytetracycline for treatment of chlamydiosis in Goffin's cockatoos. *Avian Dis* 34:1017.
- Flammer K, et al. 1991. Intramuscular and oral disposition of enrofloxacin in African grey parrots following single and multiple doses. *J Vet Pharmacol Ther* 41:359.
- Flammer K. 1992. New advances in avian therapeutics. *Proc Ann Conf Assoc Avian Vet*, pp 14–18.
- Flammer K. 1994. Antimicrobial therapy. In: Ritchie BW, et al., eds. *Avian Medicine: Principles and Applications*. Boca Raton, FL: Wingers, 434–456.
- Flammer K. 1996. Fluconazole in psittacine birds. *Proc Ann Conf Assoc Avian Vet*, pp 203.
- Flammer K, et al. 2001. Plasma concentrations of doxycycline in selected psittacine birds when administered in water for potential treatment of *Chlamydophila psittaci* infection. *J Avian Med Surg* 15:276.
- Flammer K, et al. 2002. Plasma concentrations of enrofloxacin in psittacine birds offered water medicated with 200 mg/L of the injectable formulation of enrofloxacin. *J Avian Med Surg* 16:286.
- Flammer K, et al. 2003. Assessment of plasma concentrations of doxycycline in budgerigars fed medicated seed or water. *J Amer Vet Med Assoc* 223:993.
- Flammer K. 2005. Administration strategies for delivery of enrofloxacin. *Proc Ann Conf Assoc Avian Vet*, in press.
- Frazier DL, et al. 1995. Pharmacokinetic considerations of the renal system in birds. Part I, Anatomic and physiologic principles of allometric scaling. Part II, Review of drugs excreted by renal pathways. *J Avian Med Surg* 9:92 and 104.
- Goldstein DL, et al. 2000. Renal and extrarenal regulation of body fluid composition. In: Whittow GC, ed., *Sturkie's Avian Physiology*, 5th edition. San Diego, CA: Academic Press, 265.
- Gronwall R, et al. 1989. Pharmacokinetics of amikacin in African gray parrots. *Am J Vet Res* 50:250.
- Jakoby JR, Gylstorff I. 1983. Comparative research on the medicated treatment of psittacosis. *Berl Munich Tierarztl Wocheschr* 96:261.
- Kollias GV, et al. 1986. The use of ketoconazole in birds: preliminary pharmacokinetics and clinical applications. *Proc Ann Conf Assoc Avian Vet*, 103.
- Locke D, et al. 1982. Pharmacokinetics and tissue concentrations of tylosin in selected avian species. *Am J Vet Res* 43:1807.
- Lumeij JT, et al. 1995. Plasma and tissue concentrations of itraconazole in racing pigeons (*Columba livia domestica*). *J Avian Med Surg* 9:32.
- Moore RP, et al. 2001. Diagnosis, treatment, and prevention of megabacteriosis in the budgerigar (*Melopsittacus undulatus*). *Proc Ann Conf Assoc Avian Vet*, 161–163.
- Orosz SE, et al. 1996. Pharmacokinetic properties of itraconazole in blue-fronted Amazon parrots (*Amazona aestiva aestiva*). *J Avian Med Surg* 10:168.
- Orosz SE, et al. 2000. Pharmacokinetics of amoxicillin plus clavulanic acid in blue-fronted Amazon parrots (*Amazona aestiva aestiva*). *J Avian Med Surg* 14:107.
- Quesenberry KE. 1998. Avian antimicrobial therapeutics. In: Jacobson ER, Kollias GV, eds. *Exotic Animals*. New York: Churchill Livingstone, 177.
- Powell FL. 2000. Respiration. In: Whittow GC, ed, *Sturkie's Avian Physiology*, 5th edition. San Diego, CA: Academic Press, 233.
- Powers LV, et al. 2000. Preliminary investigation of doxycycline plasma concentrations in cockatiels (*Nymphicus hollandicus*) after administration by injection or in water or feed. *J Avian Med Surg* 14:23.
- Ramsay EC, et al. 1993. Pharmacokinetic properties of gentamicin and amikacin in the cockatiel. *Avian Dis* 37:628.
- Schroeder EC, et al. 2001. Pharmacokinetics of ticarcillin and amikacin in blue-fronted Amazon parrots (*Amazona aestiva aestiva*). *J Avian Med Surg* 11:260.
- Schuetz S, et al. 2001. Pharmacokinetic and clinical studies of the carbapenem antibiotic, meropenem, in birds. *Proc Ann Conf Assoc Avian Vet*, 183.
- Teare JA, et al. 1985. Pharmacokinetics of a long-acting oxytetracycline preparation in ring necked pheasants, great horned owls and Amazon parrots. *Am J Vet Res* 46:2639.
- Tell L, et al. 1998. Pharmacokinetics of ceftiofur sodium in exotic and domestic avian species. *J Vet Pharmacol Ther* 21:85.

Antimicrobial Drug Use in Rodents, Rabbits, and Ferrets

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Due to their small space requirements and ease of care, small mammals are increasingly popular pets, and are consequently more often seen as veterinary patients. More so than with traditional species, treating these small mammals requires knowledge of their inherent biology and physiology in order to prevent antimicrobial toxicities. Correct antimicrobial treatment in these species should be based on the animal's overall health and nutritional status, duration (acute vs. chronic) of the infection, and organ system affected. Small mammals presented to the clinic commonly have microbial diseases secondary to husbandry or nutritional problems. For example, infrequent cage changes in small rodents can result in increased ammonia levels and subsequent mucous membrane irritation, making these tissues susceptible to microbial colonization. Nutritionally unbalanced diets can debilitate the animals' immune systems and predispose them to bacterial colonization. Guinea pigs not receiving their required daily vitamin C ration may present to the clinic with subclinical hypovitaminosis C. Optimizing husbandry conditions and nutrition is as important as choosing the correct antibiotic for treatment of microbial diseases. In some instances, additional procedures may be needed for effective treatment. For example, rabbit abscesses tend to be caseous and encapsulated with thick walls. Effective treatment requires concurrent surgical excision in addition to antibiotic therapy.

In laboratory animal medicine, production and maintenance of genetically modified rodents may necessitate adding clinical treatments to a primarily herd health approach provided to rodent colonies. Replacing unique constructs when they become sick may no longer be an option. Correct choice of antibiotics in these constructs is dependent on minimizing research interference and the animal's associated construct.

Whenever possible, practitioners should attempt microbial identification and sensitivity testing of the inciting agent. Drug safety, dosage, and route of administration are also important considerations when choosing the correct antibiotic. Indiscriminate use of antibiotics in these species should be avoided since inappropriate antimicrobial therapy contributes to the development of antibiotic-resistant strains of bacteria. In addition, many of the smaller species may not endure an additional treatment regime without jeopardizing their survival.

The following sections discuss proper antimicrobial selection for treating common diseases of rabbits, rodents, and ferrets. Due to their increased popularity as pets, the hedgehog (*Atelerix albiventris*) and the sugar glider (*Petaurus breviceps*) are also included in this chapter. The tables are not an exhaustive list of microbial diseases of these species, but rather a summarized list. Readers are encouraged to look elsewhere for more details on successful disease management in these species.

Special attention should be taken when treating genetically engineered rodents, as the information provided may not apply to all genetically engineered animals. Laboratory animal veterinarians must consult with the principal investigator and current scientific literature prior to initiating treatment of genetically engineered rodents or any other species used in research.

Antimicrobial Toxicity

Their dependence on microbial hindgut fermentation to obtain adequate nutrition makes rabbits and rodents more susceptible to antimicrobial toxicity.

Rabbits, guinea pigs, chinchillas, and hamsters are susceptible to antibiotic-associated enterotoxemia, whereas rats, mice, and possibly gerbils are relatively resistant. Primary factors involved in the development of dysbiosis and enterotoxemia include the use of narrow-spectrum antibiotics and the presence of pathogenic bacteria within the GI tract. Antimicrobial treatment can cause gastrointestinal flora alterations, disrupting normal microbial composition and contributing to dysbiosis. Drugs such as penicillin, ampicillin, clindamycin, lincomycin, and to a lesser extent, erythromycin and cephalosporins can induce dysbiosis. Guinea pigs may be more susceptible to dysbiosis with tetracycline administration than any of the other species. Pathogenic bacteria such as *Clostridium* spp. and *E. coli* can be found in small numbers in normal animals. The disruption of normal gastrointestinal flora can promote the overgrowth of pathogenic bacteria, if these are present in the GI tract. In rabbits, *Clostridium spiriforme* overgrowth has been implicated as the primary causative agent, producing iota toxin and eventually causing enteritis, enterotoxemia, and death. In guinea pigs, *Clostridium difficile* can cause enteritis, enterotoxemia, and death when their primarily Gram-positive gut flora is disrupted. Other factors that can increase the potential for dysbiosis are the animal's age, current diet, and stress level. Oral antibiotics tend to induce more diarrhea than injectable antibiotics. Aminoglycosides, chloramphenicol, fluoroquinolones, metronidazole, and sulfonamides are less likely to induce dysbiosis.

The toxic antimicrobial effects in small rodents are not limited to enterotoxemia. For example, deaths attributable to direct toxic effects of streptomycin and dihydrostreptomycin on the cardiovascular system, have been reported in mice, hamsters, guinea pigs, and gerbils (Wightman et al., 1980; Carpenter, 1996). Procaine toxicity associated with procaine penicillin G has been reported in mice, guinea pigs, and rabbits (Morris, 1995). Lymphoid tissue involution and impaired immune response was reported in rabbits receiving chronic tetracycline treatment (Stetsenko et al., 1981). As with other species, nephrotoxicity can occur with aminoglycoside administration. Warm fluids to maintain hydration are recommended when treating older or debilitated animals with aminoglycosides.

In the laboratory animal setting, irreplaceable genetically engineered rodent lines complicate antibiotic

selection. Their altered genetic background may adversely affect drug metabolism pathways or gene activity. Careful discussion of antibiotic choice with the primary investigator and other research team members is essential. It is judicious to "test-treat" a subset of animals before treating the larger population. Animals should be observed for signs of toxicity throughout treatment.

Ferrets' gastrointestinal physiology makes them relatively resistant to antimicrobial toxicity. Antimicrobial toxicity in hedgehogs (*Atelerix albiventris*) and sugar gliders (*Petaurus breviceps*) has not been reported.

Veterinarians treating rabbits for meat production must inform clients regarding regulations governing antimicrobial administration. Approved drug formulations and withdrawal times must be followed.

Drug Dosages

The majority of recommended dosages used in rabbits, rodents, and ferrets are empirical, extrapolated from dosages used in other species and confirmed by clinical success during their use. The vast array of published information frequently lacks pharmacokinetic data, creating additional challenges when treating these species. The dosages provided must be used with caution, especially when pharmacokinetic data are not available. Veterinarians must take into consideration the anatomy, physiology, and pharmacobiology of the species being treated. Some drugs may be ineffective in some animals at the recommended dosages. For example, tetracycline in drinking water was a treatment used in rabbits for years until a pharmacokinetic study indicated this treatment did not achieve therapeutic concentrations (Percy and Black, 1988). Some drugs may be toxic; a trial with intramuscular penicillin at 40,000 IU/kg for the treatment of *Treponema* in rabbits indicated that this dose may cause death (Harkness and Wagner, 1995).

Extrapolation of drug dosages between species through allometric scaling can be used when a dosage cannot be obtained from pharmacokinetic data. Several approaches to interspecies scaling are available (Morris, 1995). Although allometric scaling assumes no interspecies metabolic differences, particularly in smaller species, it is considered an acceptable method for estimating drug dosages.

Several published drug formularies including rab-

bits, rodents, ferrets, and other small mammals provide valuable dosage information for the laboratory animal and private practitioner. Tables 37.2 to 37.13 present drug choices and dosages considered safe and effective for the treatment of common microbial diseases. Since most antimicrobials used in these species are considered extra-label, veterinarians should inform their clients of their extra-label use, exercise caution, and use professional judgment when treating these animals.

Routes of Administration

The route of administration of any antimicrobial will depend on the animal's health status, location of infection, temperament, and the number of animals being treated. Most drug formulations are not licensed for use in these animals. Available formulations are often too concentrated, requiring appropriate dilution for accurate dosing and to minimize tissue damage. If available, the same diluent used in the original formulation should be used. Routes of administration used in rodents, rabbits and ferrets are subcutaneous, intraperitoneal, intramuscular, intravenous, oral, and topical. All routes have unique challenges, depending on the species being treated. The subcutaneous route is preferred on all small mammals because it is quick, easy, and can frequently be performed by one person. A relatively large amount of fluid can be injected in one location with minimal stress. Most small mammals presented to the clinic with systemic disease frequently have some degree of anorexia and dehydration. Subcutaneous administration provides an efficient way to provide fluid replacement with warm crystalloid solutions in conjunction with antibiotic therapy. Animals can be restrained by firmly pulling the loose skin on their dorsal cervical region with one hand while injecting with the other hand. Rabbits, chinchillas, and guinea pigs can be safely restrained by wrapping them in a towel and administering the medication under the loose skin between the shoulder blades.

The intraperitoneal route is another easy and effective means to administer antimicrobials in small mammals. Relatively large volumes of fluids can be given and the large surface area and increased vascularity provides rapid absorption. Small mammals can be restrained by securely grasping the loose skin in the

dorsal aspect of the neck and turning them upside down to expose the ventral aspect of the abdominal area.

Due to the small muscle mass and increased potential for tissue damage, intramuscular injection is strongly discouraged in small mammals. Rabbits, ferrets, and guinea pigs can be injected in the muscles of the lumbar area or in the hind legs (quadriceps or gluteals). Care must be taken not to inject close to the sciatic nerve, which runs immediately caudal to the femur, or lameness and autophagia can occur.

In small mammals, most IV administration techniques require either exceptional proficiency or anesthesia, and are thus not recommended for routine use. In rats, mice, and gerbils, the lateral tail vein and lateral saphenous vein can be used; the femoral vein can be used in hamsters. The lateral saphenous and cephalic veins may be used in chinchillas and guinea pigs. In the rabbit and ferret, the jugular, cephalic, and saphenous veins can be used with minimal effort. The marginal ear vein may also be used in rabbits and guinea pigs. In weak or dehydrated animals, the intraosseous route may be used. A catheter can be placed in the proximal head of the femur for the administration of antimicrobials and warm fluids. The reader is encouraged to read Hiller and Quesenberry for more detailed information on specific injection techniques.

Oral administration can be used when the number of animals being treated is too large to make individual treatments cost effective, handling of the animal causes excessive stress, or when appetite has not been affected. Antimicrobials added to the water must be soluble, stable, and absorbable after consumption. It is important to note that differences in water intake, appetite, body weight, and age make accurate dosage impossible when treating a large number of animals via medicated food or water. Adding an antibiotic to the food or water can also alter the flavor and affect consumption. The animal's current diet must be taken into account. Animals in controlled environments and fed dry rations will consume more water than animals offered fresh fruit and vegetables. Also, interspecies differences must be taken into account. For example, gerbils drink little or no water, whereas adult guinea pigs may drink large amounts in one day. Average daily food and water consumption for selected species can be found in Table 37.1. Table 37.3 provides recommended dosages for drug administration in food and water.

Table 37.1. Daily food and water intake per 100 g body weight.

Species	Food (Dry Weight, g)	Water (ml)
Mouse	12-18	15
Hamster	8-12	8-10
Gerbil	5-8	4-7
Rat	5-6	10-12
Guinea pig	6	10-12
Hedgehog	a	a
Sugar gliders	b	b
Chinchilla	3-6, ad lib hay	c
Rabbit	5, ad lib hay	5-10 ml
Ferret	4.3 g	7.5-10

^aCurrently undetermined, as hedgehog diets in captivity are still experimental. For diet suggestions see Smith (1999).

^bCurrently undetermined. For diet suggestions see Johnson-Delaney (2000).

^cData unavailable.

Direct dosing is preferred when animals can be individually treated. Drugs can be hidden in treats such as a piece of fruit, cooked sweet potato, or other palatable food. Alternatively, they can be compounded by pharmacies to a specific concentration and flavor for direct administration. Proper technique is essential when drugs are administered by direct oral route or aspiration can occur. Rabbits, guinea pigs, and chinchillas can be gently restrained or wrapped in a towel while a syringe with the medication is placed behind the incisors. The licking response can be stimulated if drug is given slowly, facilitating oral drug administration. For small rodents and ferrets, firmly pulling the loose skin on their dorsal cervical region will pull the lips back facilitating oral administration.

Due to their fastidious grooming habits, topical antimicrobial ointments should be used with caution. Dysbiosis can occur if these are ingested in significant amounts. They may be of therapeutic value if used in areas not readily accessible for grooming. Creams, ointments, or drops containing steroids should be used with caution in rodents because these species are particularly susceptible to their toxic side effects. Nebulization of antibiotics may be of value, especially when treating respiratory tract diseases.

Local antimicrobial delivery systems such as antibiotic impregnated polymethylmethacrylate (PMMA) beads have been used to treat abscesses and experimentally induced osteomyelitis in rabbits. These deliver high antibiotic concentrations into the affected

area with low systemic absorption rates. Thorough tissue debridement improves the effectiveness of local antimicrobial delivery systems.

In the hedgehog (*Atelerix albiventris*), medications can be given subcutaneously in the spiny or furred areas. The muscles of the thigh can be used for intramuscular injections; and the femoral, lateral saphenous, or cephalic veins can be used for intravenous administration. Oral administration may be successful if given in flavored suspensions or mixed with their favorite food.

As in other small mammals, intravenous injection in sugar gliders (*Petaurus breviceps*) is difficult and requires general anesthesia for safe and successful administration. Antimicrobials can be injected subcutaneously in the mid-dorsal thoracic area and intramuscularly in the epaxial muscles of the cervical and thoracic area or cranial muscles of the thigh.

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Bibliography

- Bishop Y, ed. 2001. The Veterinary Formulary, 5th ed. London: The Pharmaceutical Press.
- Carpenter JW, et al. 2005. Exotic Animal Formulary, 3rd ed. Elsevier Saunders.
- Collins BR. 1995. Antimicrobial drug use in rabbits, rodents, and other small mammals. In: Antimicrobial Therapy in Caged Birds and Exotic Pets, An International Symposium. Florida: Veterinary Learning Systems.
- Foltz CJ, et al. 1996. Evaluation of various oral antimicrobial formulations for the eradication of *Helicobacter hepaticus*. Lab Anim Sci 46:173.
- Fox JG, et al., eds. 2002. Laboratory Animal Medicine, 2nd ed. Elsevier Academic Press.
- Harcourt-Brown F. 2002. Textbook of Rabbit Medicine. Elsevier Butterworth-Heinemann.
- Harkness JE, Wagner JE. 1995. The Biology and Medicine of Rabbits and Rodents, 4th ed. Philadelphia: Williams and Wilkins.
- Hawk CT, et al. 2005. Formulary for Laboratory Animals, 3rd ed. Ames, IA: Blackwell Publishing.
- Hillyer EV, Quesenberry KE, eds. 2003. Ferrets, Rabbits and Rodents Clinical Medicine and Surgery, 2nd ed. Philadelphia: WB Saunders.
- Hoefer HL. 1994. Hedgehogs. In: Vet Clinics North Amer Small Anim Pract. Exotic Pet Medicine II. Philadelphia: WB Saunders. 24 (1):113.

- Hrapkiewicz K, et al. 1998. Clinical Laboratory Animal Medicine, 2nd ed. Ames, IA: Iowa State University Press.
- Johnson-Delaney CA. 1996. Exotic Companion Medicine Handbook for Veterinarians. Lake Worth, FL: Wingers.
- Johnson-Delaney CA. 2000. Medical Problems in Sugar Gliders. Exotic Pet Practice. 5:1.
- Laber-Laird K, et al. 1996. Handbook of Rodent and Rabbit Medicine. Oxford: Elsevier Science.
- Lipman NS, et al. 1992. Utilization of cholestyramine resin as a preventive treatment for antibiotic (clindamycin) induced enterotoxaemia in the rabbit. Lab Anim 26:1.
- Matsushita S, Suzuki E. 1995. Prevention and treatment of cilia-associated respiratory bacillus in mice by use of antibiotics. Lab Anim Sci 45:503.
- McKay SG, et al. 1996. Use of tilmicosin for treatment of pasteurellosis in rabbits. Am J Vet Res 57:1180.
- Meredith A, Redrobe S, eds. 2002. BSAVA Manual of Exotic Pets. Gloucestershire, UK: Brit Sm An Vet Assn.
- Morris TH. 1995. Antibiotic therapeutics in laboratory animals. Lab Anim 29:16.
- Percy DH, Black WD. 1988. Pharmacokinetics of tetracycline in the domestic rabbit following intravenous or oral administration. Can J Vet Res 52:5.
- Rosenthal KL. 1998. Bacterial infections and antibiotic therapy in small mammals. Suppl Compend Contin Educ Pract Vet 20 (3A):13.
- Richardson VCG. 2003. Diseases of Small Domestic Rodents. Oxford: Blackwell Science.
- Richardson VCG. 2000. Diseases of Domestic Guinea Pigs. Oxford: Blackwell Science.
- Smith AJ. 1992. Husbandry and medicine of African hedgehogs (*Atelerix albiventris*). J Small Exotic Anim Med 2:21.
- Smith AJ. 1999. Husbandry and nutrition of hedgehogs. In: Veterinary Clinics of North America, Exotic Animal Practice 2:1. Elsevier Science.
- Stetsenko ON, et al. 1981. Tetracycline effect on immune and hematopoietic systems of intact rabbits. Antibiotic 26:856.
- Streppa HK, et al. 2001. Applications of local antibiotic delivery systems in veterinary medicine. J Am Vet Med Assn 219:1.
- Wightman SR, et al. 1980. Dihydrostreptomycin toxicity in the Mongolian gerbil, *Meriones unguiculatus*. Lab Anim Sci 40:71.

Table 37.2a. Recommended dosages of antimicrobial drugs in small mammals.

Drug	Mice	Hamsters	Gerbils	Rats	Guinea Pigs
Amikacin ^a	8-16 mg/kg divided SID to TID, SC, IM	8-16 mg/kg divided SID to TID, SC, IM	8-16 mg/kg divided SID to TID, SC, IM	8-16 mg/kg divided SID to TID, SC, IM	8-16 mg/kg divided SID to TID, SC, IM
Ampicillin ^b	20-50 mg/kg BID, PO, SC	Do not use	6-30 mg/kg TID, PO	20-50 mg/kg BID, PO, SC	Do not use
Amoxicillin	100 mg/kg SID, SC	Do not use	d	150 mg/kg SID, IM	Do not use
Cephalexin	60 mg/kg BID, PO	d	d	60 mg/kg BID, PO	50 mg/kg SID, BID, PO,
Chloramphenicol	30-50 mg/kg BID, PO, SC	30-50 mg/kg BID, PO, SC	30-50 mg/kg BID, PO, SC	30-50 mg/kg BID, PO, SC	30-50 mg/kg BID, PO, SC
Chlortetracycline ^c	25 mg/kg BID, SC, IM	20 mg/kg BID, PO, SC, IM	d	10 mg/kg BID, SC, IM	c,d
Ciprofloxacin ^e	7-20 mg/kg SID, PO	7-20 mg/kg SID, PO	7-20 mg/kg SID, PO	7-20 mg/kg SID, PO	7-20 mg/kg SID, PO
Doxycycline ^c	2.5 mg/kg BID, PO	2.5 mg/kg BID, PO	2.5 mg/kg BID, PO	2.5 mg/kg BID, PO	2.5 mg/kg BID, PO ^c
Enrofloxacin ^e	5-10 mg/kg BID, PO, SC, IM	5-10 mg/kg BID, PO, SC, IM	5-10 mg/kg BID, PO, SC, IM	5-10 mg/kg BID, PO, SC, IM	5-15 mg/kg BID, PO, SC, IM
Gentamicin ^a	2-5 mg/kg SID, SC, IM	2-5 mg/kg SID, SC, IM	2-5 mg/kg SID, SC, IM	2-5 mg/kg SID, SC, IM	2-5 mg/kg SID, SC, IM
Griseofulvin ^h	25-50 mg/kg BID, PO	25-50 mg/kg BID, PO	25-50 mg/kg BID, PO	25-50 mg/kg BID, PO	25-50 mg/kg BID, PO
Metronidazole	10-40 mg/kg SID, PO	20 mg/kg BID, PO	20 mg/kg BID, PO	10-40 mg/kg SID, PO	20 mg/kg BID, PO
Tetracycline ^c	10-20 mg/kg BID, TID, PO	10-20 mg/kg BID, TID, PO	10-20 mg/kg BID, TID, PO	10-20 mg/kg BID, TID, PO	10-20 mg/kg ID, PO ^c
Trimethoprim-sulfamethoxazole	30mg/kg BID, PO	15-30mg/kg BID, PO	30mg/kg BID, PO	30mg/kg BID, PO	30mg/kg BID, PO
Trimethoprim-sulfadiazine ^f	30mg/kg SID, SC, IM	30mg/kg SID, SC, IM	30mg/kg SID, SC, IM	30mg/kg SID, SC, IM	30mg/kg SID, SC, IM
Tylosin ^{f,g}	10 mg/kg SID, PO,SC, IM	10 mg/kg SID, PO,SC,IM ^g	10 mg/kg SID, PO, SC, IM	10 mg/kg SID, PO, SC, IM	10 mg/kg SID, PO, SC, IM ^g

^aNephrotoxic; best given with fluids.^bProlonged treatment with penicillin or its derivatives may result in changes in microbial flora and diarrhea.^cMay induce dysbiosis, particularly in guinea pigs.^dData on safety, efficacy, and dosage not currently available.^eMay cause arthropathies in growing animals.^fMay cause tissue necrosis when given subcutaneously.^gToxicity has been reported in hamsters and guinea pigs.^hDo not use in pregnant animals: may cause diarrhea, leukopenia, diarrhea.

Table 37.2b. Recommended dosages of antimicrobial drugs in small mammals.

Drug	Chinchillas	Ferrets	Rabbits	Hedgehogs	Sugar Gliders ^j
Amikacin ^a	10 mg/kg SID to TID, SC, IM, IV	10-15 mg/kg SID, SC, IM	10 mg/kg BID, SC, IM	2.5-5 mg/kg BID, TID, IM	d
Ampicillin ^b	Do not use	5-10mg/kg BID, PO	Do not use	10 mg/kg BID, SC, IM	d
Amoxicillin	Do not use	10-20 mg/kg BID PO	Do not use	12.5 mg/kg PO, BID	30 mg/kg SID, PO, IM
Cephalexin	—	15-25 mg/kg BID, PO	11-22 mg/kg TID, PO	25 mg/kg TID, PO	30 mg/kg SID, PO
Chloramphenicol	50 mg/kg BID, PO	25-50 mg/kg BID, PO	25-50 mg/kg BID, PO, SC	30-50 mg/kg BID, PO, SC, IM, IV	d
Chlortetracycline ^c	50 mg/kg BID, PO	d	50 mg/kg SID, PO	d	d
Ciprofloxacin ^e	15-20 mg/kg SID, BID, PO	10-30 mg/kg SID, PO	5-20 mg/kg SID, PO	5-20 mg/kg BID, PO	10 mg/kg SID, PO
Doxycycline ^c	2.5 mg/kg BID, PO	2.5-5 mg/kg BID, PO	2.5 mg/kg BID, PO	d	d
Enrofloxacin ^e	5-10 mg/kg BID, PO, SC, IM	10-30 mg/kg SID, PO, SC, IM	5-20 mg/kg BID, PO, SC, IM	2.5-10 mg/kg BID, PO, SC, IM	5 mg/kg BID, SC, IM, PO
Gentamicin ^a	5-8 mg/kg BID-TID, SC, IM	5 mg/kg SID, SC, IM	4 mg/kg SID, SC, IM	2 mg/kg TID, SC, IM	2 mg/kg SID, SC, IM
Griseofulvin	25 mg/kg BID, PO ^f	25 mg/kg SID, PO	25 mg/kg SID, PO	50 mg/kg SID, PO	d
Metronidazole	10-20 mg/kg BID, PO	15-20mg/kg BID, PO	20 mg/kg BID, PO	20 mg/kg BID, PO	25 mg/kg BID, PO
Neomycin	15 mg/kg BID, PO	10-20 mg/kg QID, PO	30 mg/kg SID, PO	d	d
Oxytetracycline ^c	50 mg/kg BID, PO	20 mg/kg TID, PO	25 mg/kg SID, PO	25-50 mg/kg SID, PO	d
Tetracycline ^c	20 mg/kg BID, PO	25 mg/kg BID, TID, PO	50 mg/kg, BID, PO	10-20 mg/kg BID, PO	d
Trimethoprim/sulfa	15-30 mg/kg BID, PO, SC	15-30 mg/kg BID, PO, SC	15 mg/kg BID, PO	30 mg/kg BID, PO	15 mg/kg PO
Tylosin ^{f,g}	10 mg/kg BID, PO, SC, IM	10 mg/kg BID, PO, SC, IM	10 mg/kg SID, PO, SC, IM	10 mg/kg SID, PO, SC, IM	d

^aNephrotoxic; best given with fluids.

^bProlonged treatment with penicillin or its derivatives may result in changes in microbial flora and diarrhea.

^cMay induce dysbiosis, particularly in guinea pigs.

^dData on safety, efficacy, and dosage not currently available.

^eMay cause arthropathies in growing animals.

^fMay cause tissue necrosis when given subcutaneously.

^gToxicity has been reported in hamsters and guinea pigs.

^hDo not use in pregnant animals; may cause diarrhea, leukopenia.

ⁱUse with caution.

^jLow end of dosage range for cats, ferrets, or hedgehogs.

Table 37.3. Recommended dosages of antimicrobials administered in drinking water or food.

Drug	Mice	Hamsters	Gerbils	Rats	Guinea Pigs	Chinchillas	Rabbits
Chloramphenicol	0.5 mg/ml ^a	b	0.83 mg/ml	b	1 mg/ml	b	1.3 mg/m
Dimetridazole	1 mg/ml	0.5 mg/ml	0.5 mg/ml	1 mg/ml	b	0.8 mg/ml	0.2 mg/ml
Enrofloxacin ^c	0.05-0.2 mg/ml	0.1 mg/ml	0.1 mg/ml	0.1 mg/ml	0.05-0.2 mg/ml	0.05-0.2 mg/ml	0.05-0.2 mg/ml
Neomycin	2.6 mg/ml	0.5 mg/ml	2.6 mg/ml	2.6 mg/ml	b	b	0.2-0.8 mg/ml
Oxytetracycline ^d	0.4 mg/ml	0.25-1 mg/ml	0.8 mg/ml	0.4 mg/ml	1 mg/ml ^d	1 mg/ml	1 mg/ml
Sulfamerazine	1 mg/ml or 0.25 mg/g diet	1 mg/ml	0.8 mg/ml	1 mg/ml or 0.25 mg/g diet	1 mg/ml	1 mg/ml	1 mg/ml
Sulfamethazine	1 mg/ml	1 mg/ml	0.8 mg/ml	1 mg/ml	1 mg/ml	1 mg/ml	1-5 mg/ml or 5-10 mg/g diet
Sulfaquinoxaline	1 mg/ml	1 mg/ml	1 mg/ml	1 mg/ml or 0.5mg/g diet	1 mg/ml	1 mg/ml	1 mg/ml or 0.6 mg/g diet
Tetracycline ^d	2-5 mg/ml	0.4 mg/ml	2-5 mg/ml	2-5 mg/ml or 1-5mg/g diet	0.7 mg/ml ^d	0.3-2 mg/ml	1 mg/ml
Tylosin ^e	0.5 mg/ml	0.5 mg/ml ^e	0.5 mg/ml	0.5 mg/ml	0.5 mg/ml ^e	b	b

^aPer ml of drinking water.^bData on safety, efficacy, and dosage are not available.^cMay cause arthropathies in growing animals.^dMay induce dysbiosis, particularly in guinea pigs.^eToxicity has been reported in hamsters and guinea pigs.

Table 37.4. Antimicrobial treatment in mice.

Site	Clinical Signs/Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs
Skin and subcutis	Scabbing over shoulders and back, dermatitis, abscesses	<i>S. aureus</i> , <i>Streptococcus</i> spp.	Secondary to primary wounds caused by fighting or self-trauma due to acariasis.	Ampicillin; chloramphenicol; tetracyclines
	Mastitis	<i>S. aureus</i>	Lance and drain the gland in addition to antibiotics.	Ampicillin, chloramphenicol, fluoroquinolones
	Pruritus, weight loss, hyperkeratosis, alopecia (in haired mice)	<i>C. bovis</i>	Affects nude mice, other immunocompromised mice. Low mortality. Treatment not curative.	Ampicillin, penicillin
	Alopecia, erythema, crusting on face, head, neck, tail	<i>Trichophyton mentagrophytes</i>	Uncommon, zoonotic.	Griseofulvin
Respiratory	Rhinitis, dyspnea, otitis media, upper respiratory tract disease, pneumonia	<i>M. pulmonis</i>	Often concurrent with Sendai virus or CAR bacillus; decrease intracage ammonia levels.	Tylosin; fluoroquinolone; tetracyclines
	Dacryoadenitis, sneezing, dyspnea, pneumonia	<i>P. pneumotropica</i> , <i>K. pneumonia</i> , <i>B. bronchiseptica</i>	Often concurrent with Sendai virus or CAR bacillus; decrease intracage ammonia levels.	Chloramphenicol; fluoroquinolones; tylosin; aminoglycosides ^a
	Pneumonia	CAR bacillus	Primary or opportunist in association with respiratory pathogens. Genotype, age, and diet influence course and severity of disease.	Sulfamerazine; ampicillin; trimethoprim-sulfa
Gastrointestinal	Stunted growth, diarrhea, rectal prolapse, death; transmissible murine colonic hyperplasia	<i>C. rodentium</i> , <i>Clostridium piliforme</i>		Tetracyclines; neomycin; metronidazole
	Liver disease, death; chronic active hepatitis	<i>Helicobacter hepaticus</i>		See footnote ^b
	Anorexia, dehydration, diarrhea, death; Tyzzer's disease	<i>C. piliforme</i>	Concurrent fluid therapy essential.	Tetracyclines
	Anorexia, weight loss, lethargy, dull coat	<i>Salmonella enteritidis</i> , <i>S. typhimurium</i>	Zoonotic; recommend culling infected animals.	
Urogenital	Oophoritis, salpingitis, metritis, infertility, abortions	<i>M. pulmonis</i> , <i>P. pneumotropica</i> , <i>K. oxytoca</i>		Tylosin; fluoroquinolones; tetracyclines
	Urethral gland obstruction, preputial gland abscesses	<i>P. pneumotropica</i> , <i>S. aureus</i>		Chloramphenicol; fluoroquinolones; aminoglycosides ^a
CNS	Head tilt, torticollis, circling	<i>M. pulmonis</i> , <i>Streptococcus</i> spp.		Chloramphenicol; tylosin; fluoroquinolones
	Eye abscesses, conjunctivitis, panophthalmitis	<i>P. pneumotropica</i>		Tetracyclines; aminoglycosides ^a
General	Septicemia, death; mice that survive acute infection may have chronic arthritis, limb deformity, limb amputation; streptobacillosis	<i>Streptobacillus moniliformis</i>	Zoonotic potential.	Ampicillin; tetracycline
	Rough hair coat, hunched posture, inappetence, nasal and ocular discharge, arthritis	<i>C. kutscheri</i>	Antibiotic treatment not curative.	

^aAminoglycosides: systemic use of amikacin or gentamicin.

^bTreatment of choice: amoxicillin 1.5-3 mg/30g/d, metronidazole 0.69 mg/30g/d, bismuth subsalicylate 0.185 mg/30g/d, combined PO.

Table 37.5. Antimicrobial treatment in hamsters.

Site	Clinical Signs/Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs
Skin and subcutis	Cheek pouch abscesses, abscesses	<i>S. aureus</i> , <i>Streptococcus</i> spp., <i>P. pneumotropica</i> , <i>Actinomyces</i> spp.	Drain and flush; complete excision of abscess beneficial.	Chloramphenicol, tetracyclines, fluoroquinolones
	Swollen lymph nodes, lymphadenitis	<i>S. aureus</i> , <i>Streptococcus</i> spp.		Chloramphenicol, tetracyclines, fluoroquinolones
	Mastitis	Beta-hemolytic streptococci	Glands warm and swollen. Supportive treatment; self-limiting infection.	
	Alopecia, dry skin, yellow flaky seborrhea	<i>Trichophyton mentagrophytes</i>	Sometimes pruritic; improve cage ventilation.	Griseofulvin
Respiratory	Sneezing, dyspnea, upper respiratory tract disease and/or pneumonia	<i>P. pneumotropica</i> , <i>S. pneumoniae</i> , <i>Streptococcus</i> spp. CAR bacillus	Secondary to poor nutrition and husbandry.	Chloramphenicol, fluoroquinolones, tetracyclines
			Opportunist in association with respiratory pathogens.	Sulfamerazine, ampicillin, sulfonamides
Gastrointestinal	Diarrhea, stained perineum, lethargy, anorexia, rectal prolapse, proliferative ileitis	<i>Lawsonia intracellularis</i>	Especially in 3–10 week olds; difficult to treat successfully; concurrent fluid therapy essential.	Tetracyclines, fluoroquinolones, trimethoprim-sulfa
	Enteritis	<i>E. coli</i> , <i>Clostridium difficile</i>	Concurrent fluid therapy essential.	Fluoroquinolones, metronidazole, tetracyclines
	Anorexia, dehydration, diarrhea, death, Tyzzer's disease	<i>Clostridium piliforme</i>	Concurrent fluid therapy essential.	Tetracyclines
	Catarrhal enteritis in weanlings	<i>Giardia muris</i>		Metronidazole
CNS	Squinting, rubbing eye, corneal ulceration	<i>Pasteurella</i> spp., <i>Streptococcus</i> spp.	Topical treatment.	Chloramphenicol, tetracyclines

Table 37.6. Antimicrobial treatment in gerbils.

Site	Clinical Signs/Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs
Skin and subcutis	Red, crusty nares, staining on forepaws, nasal dermatitis	<i>S. aureus</i> , <i>Staphylococcus</i> spp., <i>Streptococcus</i> spp.	Secondary to irritation due to Harderian gland secretions.	Chloramphenicol, trimethoprim-sulfa, fluoroquinolones
	Midventral marking gland infection, dermatitis	<i>S. aureus</i> , <i>Streptococcus</i> spp.		Chloramphenicol, trimethoprim-sulfa, fluoroquinolones
	Alopecia, hyperkeratosis	<i>Trichophyton mentagrophytes</i> , <i>Microsporum gypseum</i>	Zoonotic.	Griseofulvin
Respiratory	Sneezing, dyspnea, weight loss	Beta-hemolytic streptococci, <i>Pasteurella pneumotropica</i> ,	Rare; concurrent therapy with oxygen, mucolytics, bronchodilators may be useful.	Fluoroquinolones, oxytetracycline, sulfonamides
Gastrointestinal	Lethargy, anorexia, diarrhea, sudden death, Tyzzer's disease	<i>Clostridium piliforme</i>	Highly susceptible.	Tetracyclines
	Diarrhea, salmonellosis symptoms, death	<i>S. enteritidis</i> , <i>S. typhimurium</i>	Zoonotic; recommend culling affected animals. Fluid therapy is essential if treatment is attempted.	Chloramphenicol, fluoroquinolones
	Enteritis, diarrhea, dehydration	<i>E. coli</i>		Chloramphenicol, fluoroquinolones

Table 37.7. Antimicrobial treatment in rats.

Site	Clinical Signs/Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs
Skin and subcutis	Abrasions/ulcerations over shoulders and back; dermatitis	<i>S. aureus</i>	Secondary to primary wounds caused by self-trauma.	Ampicillin, chloramphenicol, tetracyclines
	Abscesses, furunculosis, mastitis	<i>P. pneumotropica</i> , <i>K. pneumoniae</i>	Secondary opportunist; drain and flush abscesses.	Chloramphenicol, fluoroquinolones, aminoglycosides ^a
	Mastitis	<i>P. pneumotropica</i> , <i>S. aureus</i>	Hot compresses, drainage.	Chloramphenicol, fluoroquinolones, aminoglycosides ^a
Respiratory	Alopecia, pruritus	<i>Microsporum spp.</i>	Zoonotic.	Griseofulvin
	Sneezing, dyspnea, vestibular disease, depression, chromodacryorrhea; upper respiratory tract disease and/or pneumonia	<i>M. pulmonis</i>	Common; improve nutrition, husbandry; decrease intracage ammonia levels.	Combination enrofloxacin 10 mg/kg & doxycycline 5 mg/kg beneficial; tylosin
	Serosanguinous to mucopurulent nasal discharge, rhinitis, conjunctivitis, otitis media	CAR bacillus <i>S. pneumoniae</i> ,	Synergistic interaction between <i>P. pneumotropica</i> coronavirus.	Sulfamerazine, ampicillin Combination enrofloxacin 10 mg/kg & doxycycline 5 mg/kg beneficial; tylosin
Gastrointestinal	Diarrhea, dehydration, anorexia, death; Tyzzer's disease	<i>Clostridium piliforme</i>		Tetracyclines
	Diarrhea, dry coat, unthriftiness, death	<i>Salmonella enteritidis</i>	Zoonotic; recommend culling infected animals.	
Urogenital	Infertility, oophoritis, salpingitis, metritis, pyometra	<i>M. pulmonis</i>		Tylosin, fluoroquinolones, tetracyclines
	Preputial gland abscesses	<i>S. aureus</i> , <i>P. pneumotropica</i>		Chloramphenicol, fluoroquinolones
CNS	Head tilt, circling, torticollis, otitis interna	<i>M. pulmonis</i> ± secondary bacterial invaders		Fluoroquinolones, chloramphenicol, tylosin
General	Bacteremia, septicemia, multi-organ abscessation and infarction	<i>S. pneumoniae</i>		Ampicillin
	Ruffled fur, hunched posture, porphyria, mucopurulent ocular and nasal discharge, dyspnea	<i>C. kutcheri</i>	Antibiotic treatment not curative.	

^aSystemic amikacin or gentamicin.

Table 37.8. Antimicrobial treatment in guinea pigs.

Site	Clinical Signs/Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs
Skin and subcutis	Enlarged lymph nodes, cervical lymphadenitis	<i>S. zooepidemicus</i>	May cause septicemia; complete surgical excision of infected lymph nodes beneficial.	Chloramphenicol, fluoroquinolones
	Wet chin, "slobbers"	Oral microflora (e.g., <i>S. aureus</i>)	Secondary to malocclusion.	Chloramphenicol, trimethoprim-sulfa
	Mastitis	<i>Klebsiella</i> , <i>Staph. spp.</i> , <i>Strep. spp.</i> , <i>Pasteurella spp.</i> , <i>E. coli</i> , <i>Proteus spp.</i>	Hot compresses, milk out infected glands.	Amikacin, fluoroquinolones, sulfonamides
	Swollen, ulcerated foot; ulcerative pododermatitis; osteomyelitis	<i>S. aureus</i> , <i>Actinomyces spp.</i>	Secondary to trauma.	Chloramphenicol, fluoroquinolones
	Circular areas of alopecia, pruritus	<i>Trichophyton mentagrophytes</i> , <i>Microsporum canis</i>	Zoonotic, transmitted by direct contact and fomites.	Griseofulvin
Respiratory	Rhinitis, tracheitis, otitis media, nasal and ocular discharge, upper respiratory tract disease and/or pneumonia	<i>B. bronchiseptica</i>	Commonly carried by dogs and rabbits; some success with <i>Bordetella bacterins</i> .	Amikacin, fluoroquinolones, chloramphenicol
Gastrointestinal		<i>S. pneumoniae</i> , <i>S. zooepidemicus</i> , <i>K. pneumoniae</i>		Amikacin, fluoroquinolones, chloramphenicol
	Anorexia, diarrhea, enteritis, death	<i>C. difficile</i> , <i>S. typhimurium</i> , <i>S. enteritidis</i> , <i>E. coli</i> , <i>Yersinia pseudotuberculosis</i> , <i>Pseudomonas aeruginosa</i> , <i>Listeria monocytogenes</i>	Concurrent fluid therapy. essential; amikacin best for <i>P. aeruginosa</i>	Chloramphenicol, aminoglycosides ^a
	Anorexia, ascites, diarrhea, death, Tyzzer's disease	<i>Clostridium piliforme</i>	Recent weanlings, predisposed by crowding, poor sanitation.	Tetracyclines
	Diarrhea, coccidiosis	<i>Eimeria caviae</i>	Most common in juveniles.	Sulfonamides
	Failure to gain, weight loss, diarrhea, death; cryptosporidiosis	<i>Cryptosporidium wrairi</i>	In humans, newer macrolides such as roxithromycin and azithromycin have shown some efficacy.	
Urogenital	Endometritis, abortions, stillbirths	<i>B. bronchiseptica</i> , <i>Streptococcus spp.</i>		Chloramphenicol, aminoglycosides ^a
	Cystitis	<i>S. pyogenes</i> , <i>Staphylococcus spp.</i> , fecal coliforms	Urinary calculi often present.	Trimethoprim-sulfa; fluoroquinolones
Eye	Ocular discharge, conjunctivitis	<i>C. psittaci</i> , <i>B. bronchiseptica</i> , <i>S. pneumoniae</i>	Topical treatment; rule out concurrent hypovitaminosis C.	Tetracyclines, fluoroquinolones
General	Anorexia, soft stools, dyspnea, splenitis, hepatitis, lymphadenitis, septicemia, death	<i>S. typhimurium</i> , <i>S. enteritidis</i>	Zoonotic; recommend culling infected animals.	

^aSystemic amikacin or gentamicin.

Table 37.9. Antimicrobial treatment in ferrets.

Site	Clinical Signs/Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs
Skin and subcutis	Dermatitis, abscesses	<i>Staphylococcus</i> spp., <i>Streptococcus</i> spp., <i>Corynebacterium</i> spp., <i>Pasteurella</i> spp., <i>Actinomyces</i> spp., <i>E. coli</i>	Secondary to bite wounds; debride and flush.	Ampicillin, chloramphenicol, fluoroquinolones
	Cervical masses with sinus tracts containing thick yellow-green pus, actinomycoses	<i>Actinomyces</i> spp.	Debride and flush.	Ampicillin, tetracyclines
	Skin black, dam ill, dehydrated, acute gangrenous mastitis	<i>Staphylococcus</i> spp., coliforms	Immediate surgical excision of infected gland; contagious between dams.	Ampicillin, fluoroquinolones
	Glands firm, scarred, not painful or discoloured, chronic mastitis	<i>Staphylococcus</i> spp., <i>E. coli</i> .	Contagious between dams; appears insidiously when kits 3 weeks old.	Treatment generally ineffective
Respiratory	Dyspnea, cyanosis, upper respiratory tract disease and/or pneumonia	<i>S. zooepidemicus</i> , <i>S. pneumoniae</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> , <i>B. bronchiseptica</i> , <i>L. monocytogenes</i> <i>Pneumocystis carinii</i> <i>S. pneumoniae</i> , <i>S. zooepidemicus</i> , <i>K. pneumoniae</i>	Secondary to influenza virus, respiratory syncytial virus, canine distemper virus.	Ampicillin, tetracyclines, fluoroquinolones
Gastrointestinal	Dental tartar, gingivitis, periodontal disease	Multiple etiologies	Improve diet, dentistry.	Trimethoprim-sulfa Amikacin, fluoroquinolones, chloramphenicol Metronidazole
	Diarrhea, wasting, black, tarry stool, anemia, gastritis, gastric, duodenal ulceration, <i>H. mustelidae</i> gastritis	<i>Helicobacter mustelae</i>	Rule out foreign body, lymphoma, Aleutian disease, coronavirus.	See footnote ^a
	Diarrhea, wasting, tenesmus, prolapsed rectum, proliferative bowel disease	<i>Lawsonia intracellularis</i>		Chloramphenicol, tylosin
	Acute gastric distension, dyspnea, cyanosis, sudden death; gastric bloat	<i>C. perfringens</i>	Treat as for bloat in canine patients.	Metronidazole
	Fever, bloody diarrhea, lethargy; salmonellosis	<i>S. newport</i> , <i>S. typhimurium</i> , <i>S. choleraesuis</i>	Zoonotic; recommend culling infected animals.	
	Weight loss, diarrhea, vomiting, granulomatous inflammation, mycobacteriosis	<i>Mycobacterium</i> spp.	Zoonotic potential.	
	Diarrhea, coccidiosis	<i>Coccidia</i> spp.		Sulfonamides
	Diarrhea, giardiasis	<i>Giardia</i> spp.		Metronidazole
Urogenital	Straining, hematuria, cystitis	<i>Staphylococcus</i> spp., <i>Proteus</i> spp.	Urolithiasis often present.	Fluoroquinolones, ampicillin; sulfonamides

^aTreatment of choice: amoxicillin 10 mg/kg, metronidazole 20 mg/kg, and bismuth subsalicylate 17 mg/kg, combined and administered PO, BID.

Table 37.10. Antimicrobial treatment in chinchillas.

Site	Clinical Signs/Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs
Skin and subcutis	Abscesses	<i>S. aureus</i> , <i>Streptococcus</i> spp., <i>Pseudomonas</i> spp.	Secondary to primary wounds; complete surgical excision beneficial.	Chloramphenicol, tetracyclines, fluoroquinolones
Respiratory	Patches of alopecia, scales on nose, ears and feet	<i>Trichophyton mentagrophytes</i>		Griseofulvin, ketoconazole, itraconazole
	Anorexia, upper respiratory tract disease, pneumonia	<i>P. multocida</i> , <i>Bordetella</i> sp., <i>S. pneumoniae</i> , <i>P. aeruginosa</i>	Amikacin best for <i>P. aeruginosa</i> . Overcrowding, high humidity, poor ventilation are predispos- ing factors.	Amikacin, fluoroquinolones, tetracyclines
Gastrointestinal	Anorexia, decreased fecal output, diarrhea, enteritis, sudden death	<i>Y. enterocolitica</i> , <i>Clostridium</i> <i>perfringens</i> , <i>E. coli</i> , <i>Proteus</i> sp., <i>S. typhimurium</i> , <i>S.</i> <i>enteritidis</i> , <i>P. aeruginosa</i> , <i>L. monocytogenes</i> , <i>Corynebacterium</i> spp.	Concurrent fluid therapy essential; sulfonamides best for <i>L.</i> <i>monocytogenes</i> .	Aminoglycosides, fluoro- quinolones, sulfonamides
Urogenital	Diarrhea, giardiasis	<i>Giardia</i> spp.		Metronidazole
	Depression, abortions Metritis, fever, purulent vulvar discharge	<i>L. monocytogenes</i> <i>E. coli</i> , <i>Pseudomonas</i> spp., <i>Staphylococcus</i> spp., <i>Streptococcus</i> spp.	Highly susceptible.	Sulfonamides, tetracyclines Aminoglycosides, fluoro- quinolones
CNS	Depression, ataxia, convul- sions, sudden death	<i>L. monocytogenes</i>	Highly susceptible.	Trimethoprim-sulfa, tetracyclines
General	Septicemia, death	<i>Streptococcus</i> spp., <i>Enterococcus</i> spp., <i>P. multocida</i> , <i>K.</i> <i>pneumoniae</i> , <i>Actinomyces</i> spp., <i>F. necrophorum</i>		Chloramphenicol, fluoroquinolones

Table 37.11. Antimicrobial treatment in rabbits.

Site	Clinical Signs/Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs
Skin and subcutis	Abscesses	<i>P. multocida</i> , <i>S. aureus</i> , <i>Pseudomonas</i> , <i>Proteus</i> , <i>Streptococcus</i> , <i>Bacteroides</i> , other anaerobes	Can be located anywhere on the body; complete surgical excision of abscess beneficial.	Chloramphenicol, tetracyclines, fluoroquinolones
	Dermatitis	<i>S. aureus</i>	Usually secondary to poor husbandry.	Chloramphenicol, tetracyclines, fluoroquinolones
	Wet chin, dewlap; "slobbers"	<i>Pseudomonas aeruginosa</i>	May turn fur green.	Amikacin
	Ulceration of face, metacarpals, plantar metatarsals; necrobacillosis	<i>F. necrophorum</i>	Occurs under unsanitary conditions.	Metronidazole, chloramphenicol, tetracyclines
	Mastitis	<i>S. aureus</i> , <i>Pasteurella</i> spp., <i>Pseudomonas</i> spp.	Hot compresses and milk out affected glands often.	Amikacin, fluoroquinolones, chloramphenicol, tetracyclines
Respiratory	Alopecia, scaling, crusting at base of ears and muzzle	<i>Tricophyton mentagrophytes</i>	Zoonotic. Clip hair over affected area.	Griseofulvin, miconazole
	Nasal discharge, conjunctivitis, upper respiratory tract disease and/or pneumonia	<i>P. multocida</i>	Common.	Fluoroquinolones, tetracyclines, aminoglycosides ^a
		<i>B. bronchiseptica</i> , <i>S. aureus</i> , <i>Pseudomonas aeruginosa</i>	Usually secondary to <i>Pasteurella</i> .	Amikacin, fluoroquinolones, tetracyclines
Gastrointestinal ^b	Mucoid diarrhea, bloat, anorexia, borborygmus, mucoid enteropathy	<i>Lawsonia intracellularis</i>	Major cause of morbidity and mortality in 7-14 week olds.	Chloramphenicol
	Diarrhea, death; Tyzzer's disease	<i>Clostridium piliforme</i>		Tetracyclines
	Diarrhea, death; colibacillosis	<i>E. coli</i>	Especially neonates 1-14 days old and weanlings.	Sulfonamides, fluoroquinolones, amikacin
	Diarrhea, enterotoxemia, death	<i>C. spiroforme</i>	Weanlings especially susceptible.	Metronidazole, chloramphenicol
	Diarrhea, death	<i>Salmonella</i> spp., <i>Pseudomonas</i> spp.	Concurrent fluid therapy essential.	Chloramphenicol, fluoroquinolones
	Diarrhea, coccidiosis	<i>Eimeria</i> spp.	Hepatic or intestinal; improve sanitation.	Trimethoprim-sulfa
Urogenital	Reddening, edema of external genitalia to dry, scaly, slightly raised areas; venereal spirochetosis	<i>T. parvulus cuniculi</i>		Tetracyclines, chloramphenicol
	Abortion	<i>L. monocytogenes</i> , <i>P. multocida</i>		Trimethoprim-sulfa, chloramphenicol, tetracyclines
	Cystitis	<i>E. coli</i> , <i>Pseudomonas</i> spp.		Trimethoprim-sulfa, fluoroquinolones
	Orchitis, metritis	<i>P. multocida</i> , <i>S. aureus</i>		Chloramphenicol, tetracycline, gentamicin
	Polydipsia, polyuria, depression, anorexia, renal failure	<i>Leptospira</i> spp.	Contact with wild rodents. Diagnosis by serology.	Penicillin
Ocular	Clear to white discharge from one or both eyes, conjunctivitis	<i>P. multocida</i> , <i>S. aureus</i>	Treat topically; flush tear ducts.	Chloramphenicol, tetracyclines, aminoglycosides
CNS	Head tilt, nystagmus, torticollis	<i>P. multocida</i>	Usually due to otitis media.	Chloramphenicol, fluoroquinolones
	Ataxia, torticollis, tremors, convulsions	<i>Encephalitozoon cuniculi</i>	Diagnosis by clinical signs and serology.	Fenbendazole, albendazole, tetracyclines
General	Lethargy, anorexia, pyrexia, septicemia	<i>P. multocida</i> , <i>L. monocytogenes</i>		Fluoroquinolones, aminoglycosides, tetracyclines, chloramphenicol

^aSystemic amikacin or gentamicin.

^bWhere applicable, supportive therapy should include IV or IO fluids, cisapride or metoclopramide, high fiber diet, metronidazole 20 mg/kg BID; cholestyramine at 2 g per 20 ml water BID by gavage may bind bacterial toxins.

Table 37.12. Antimicrobial treatment in hedgehogs (*Atelerix albiventris*).

Site	Clinical Signs/Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs
Skin and subcutis	Dermatitis, abscesses	<i>S. aureus</i> , <i>Streptococcus</i> spp., various external parasites	Secondary to acariasis or bite wounds; concurrent antiparasitic therapy should be considered.	Ampicillin, chloramphenicol
	Granulomatous subcutaneous lesions, lymphadenitis	<i>Mycobacterium</i> spp.	Zoonotic potential.	Treatment unknown
	Crusting, non-pruritic dermatitis on face and pinnae	<i>Trichophyton erinacei</i>		Griseofulvin
Respiratory	Anorexia, catarrhal rhinitis, upper respiratory tract disease, pneumonia	<i>B. bronchiseptica</i> , <i>P. multocida</i> , <i>Corynebacterium</i> spp.	Cytomegalovirus, verminous pneumonia may also be involved.	Fluoroquinolones, Trimethoprim-sulfa, tetracyclines
Gastrointestinal	Gingivitis, periodontitis	Multiple etiologies	Improve diet; extractions may be beneficial.	Metronidazole
Urogenital General	Anorexia, diarrhea, weight loss, enteritis, salmonellosis	<i>Salmonella tilene</i>	Concurrent fluid therapy essential, zoonotic potential.	Chloramphenicol, fluoroquinolones
	Diarrhea, coccidiosis	Unspecified		Sulfonamides
	Diarrhea, giardiasis	<i>Giardia</i> spp.		Metronidazole
	Leptospirosis	<i>Leptospira</i> spp.	Zoonotic potential.	Ampicillin, tetracyclines
	Septicaemia, death, tularemia	<i>Francisella tularensis</i>		Aminoglycosides ^a
	Chronic wasting, submandibular lymphadenopathy, acute fatal septicemia	<i>Yersinia pseudotuberculosis</i>	Zoonotic potential.	Aminoglycosides ^a , fluoroquinolones, tetracyclines
	Q fever ^b	<i>Coxiella burnetii</i>	Zoonotic potential.	Tetracyclines, fluoroquinolones

^aSystemic amikacin or gentamicin.^bReported in wild African hedgehogs.**Table 37.13.** Antimicrobial treatment in sugar gliders (*Petaurus breviceps*).

Site	Clinical Signs/Diagnosis	Organism	Suggested Drugs
Gastrointestinal	Intermittent diarrhea	<i>Giardia</i>	Metronidazole
General	Depression, loss of appetite, weight loss	<i>Pasteurella multocida</i> , <i>Clostridium piliforme</i>	Chloramphenicol, fluoroquinolones

Antimicrobial Drug Use in Reptiles

Elliott R. Jacobson

Infectious diseases are important causes of illness and mortality in captive reptiles (Jacobson, 1980; Austwick and Keymer, 1981; Clark and Lunger, 1981; Cooper, 1981; Hoff et al., 1984; Jacobson, 1987; Jacobson, 1999). Although not as well documented, certain infectious diseases have been seen in wild populations of reptiles. For instance, die-offs of American alligators, *Alligator mississippiensis*, have been associated with *Aeromonas hydrophila* infections (Shotts et al., 1972). While a wide variety of bacteria have been incriminated as either primary or secondary pathogens of reptiles, it appears that infections caused by Gram-negative bacteria are most common. *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Providencia rettgeri*, *Morganella morganii*, *Salmonella arizonae*, *Serratia* spp., and *Klebsiella oxytoca* are prominent among the microorganisms isolated from healthy and ill captive reptiles. These bacteria become invasive when conditions that decrease the resistance of the host select for pathogenic organisms (Cooper, 1981). They also follow primary viral infection, such as ophidian paramyxovirus pneumonia (Jacobson, 1992) and herpesvirus stomatitis of tortoises (Origi et al., 2004).

Some species of reptiles seem to be particularly prone to infection with specific bacteria. *Neisseria iguanae* has been isolated from the oral cavity and bite wounds of the green iguana, *Iguana iguana* (Barrett et al., 1994) and *Mycoplasma agassizii* is a causative agent of a chronic upper respiratory disease in the desert tortoise (*Gopherus agassizii*), gopher tortoise (*Gopherus polyphemus*), and other tortoises (Jacobson et al., 1991; Brown et al., 1995; Brown et al., 1999). *Mycoplasma crocodyli* has been identified as the cause of polyarthritis in Nile crocodiles (*Crocodylus niloticus*) in Zimbabwe (Kirchoff et al., 1997; Mohan et al., 1995), and *M. alligatoris* was identified as the causative

agent of arthritis and pneumonia in the American alligator (Brown et al., 2001; Clippinger et al., 2000).

Some pathogens such as *Chlamydia*, while being seen in reptiles (Homer et al., 1994; Jacobson and Telford, 1990; Jacobson et al., 1989; Jacobson et al., 2002; Soldati et al., 2004), appear to be underreported. This is probably because they have been missed rather than being uncommon in reptiles. Special staining is needed to demonstrate *Chlamydia* in tissue section.

Mycotic infections are commonly seen in all major groups of captive reptiles, with the integumentary and respiratory system most often involved (Austwick and Keymer, 1981; Migaki et al., 1984). While *Microsporum* and *Trichophyton*, which cause dermatomycotic infections in mammals, appear to rarely affect reptiles, others such as fusariosis, geotrichosis, phycomycosis, and chromomycosis are common. The *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) has surfaced as an important fungal pathogen of reptiles (Pare et al., 1997; Pare et al., 2003; Bertelsen et al., 2005). Predisposing factors such as suboptimal cage temperature and foul environmental conditions are generally involved.

Antimicrobial therapy is an important component in medical management of reptiles that are affected with bacterial or mycotic disease. Selecting the appropriate antimicrobial agent for reptiles is more difficult than for mammals because of the diversity of their behavioral, anatomic, and physiological features. Given that relatively few pharmacokinetic (PK) studies of antimicrobial agents have been performed in a small number of reptilian species, dosages are either extrapolated from one species to others or are empirically derived. Still, the database is slowly increasing as new PK studies are published each year.

Antimicrobial Drug Selection

When selecting an antimicrobial drug to treat an infectious disease in a reptile, identification of the causative microorganism is of primary importance. If a lesion is present, a biopsy specimen should be obtained for cytologic and histologic examination in addition to collecting a swab specimen for culture. This approach is required to interpret the significance of the microorganism(s) cultured (Tables 38.1 to 38.3). The isolation of a microbe from a lesion may not necessarily mean that the causative agent has been identified. Contaminants and secondary invaders may be recovered instead of the primary agent. Some pathogens such as *Chlamydia* or *Chlamydophila* are extremely difficult to isolate and immunohistochemical and molecular techniques such as polymerase chain reaction (PCR) amplification can be used to detect their presence (Bodetti et al., 2002; Jacobson et al., 2004). In a retrospective study of 90 reptiles with granulomatous lesions, both immunoperoxidase staining with a monoclonal antibody against chlamydial LPS antigen and PCR amplification of a small 92-bp fragment of the 23S rRNA gene were used to detect chlamydiae in these tissues (Soldati et al., 2004). Identification of such pathogens is essential when deciding the most appropriate antimicrobial. In reptiles suspected of being septic, blood culture should be performed. Techniques for collecting specimens from reptiles have been described elsewhere (Jacobson, 1992). Certain bacteria such as *Clostridium* have been cultured from the blood of clinically healthy reptiles (Hanel et al., 1999) and culture results must be interpreted in the context of other health assessment tests. Bacterial contamination is common when percutaneous blood samples are collected for culture. Collecting a truly uncontaminated ante-mortem blood sample from an animal is far more difficult than it appears to be.

For bacteria that can be readily cultured, following isolation and identification, the minimum inhibitory concentrations (MICs) of potentially effective antimicrobial agents should be determined. Selection of the most appropriate antimicrobial agent will depend upon the results of quantitative susceptibility tests in conjunction with the following considerations: site of infection and type of lesion; pharmacodynamic properties and PK characteristics of antimicrobial agents in the species of reptile affected; and the clinical condition, health status, size, temperament, and immune

status of the ill reptile. The cost of the antimicrobial may be another factor influencing selection. The clinician should select an antimicrobial drug that will attain therapeutic concentrations at the site of infection. The dosage form and route of administration will be largely governed by the species of reptile. Antimicrobials that can be administered once every few days have value in treating venomous and fractious reptiles, compared to drugs that must be administered daily.

Even fewer PK studies have been done with antifungal drugs in reptiles (Table 38.4). Except for integumentary lesions that are easily biopsied, determining the antemortem presence of fungi in internal organs is difficult. In most cases, systemic fungal infections are identified following necropsy.

There are certain biological features of reptiles that influence response to treatment. The development of granulomatous inflammation is common in reptiles affected with bacterial and fungal diseases (Montali, 1988). The causative organism may be within the necrotic center of heterophilic granulomas, or within histiocytes (macrophages) in histiocytic granulomas. This limits penetration of most antimicrobial agents to these sites of infection. Granulomatous subcutaneous masses should be removed surgically to improve the chances of a positive therapeutic outcome.

A unique anatomic feature of chelonians is the shell. This is composed of an outer keratinized epidermis overlying dermal cartilage and bone. Ribs and vertebrae are incorporated into the shell. The dermal bone is highly vascularized and is a metabolically active structure. Chelonians are dosed based on their entire body weight. Lesions are commonly seen in the chelonian shell and treatment is often prolonged and difficult to assess. No studies have been done to assess penetration of antimicrobials into the shell.

Another unique feature of some reptiles is the spectacle, which embryologically represents a fusion of the upper and lower eyelids that has become transparent. It is located over the cornea in all snakes with eyes and in some lizards. Infections of the subspectacular space have been reported (Millichamp et al., 1983), and topically applied antimicrobial agents do not appear to penetrate this barrier. In treating reptiles with subspectacular infections, a wedge should be excised from the lower half of the spectacle and the appropriate antimicrobial drug applied directly onto the globe and within the space.

Of the 7,500 species of reptiles, PK characteristics of

Table 38.1. Antimicrobial drug selection in chelonian infections.

Site or Type	Diagnosis	Common Infecting Organisms	Suggested Drugs
Skin, shell, subcutis	Epidermitis/dermatitis	<i>Citrobacter freundii</i> <i>Serratia</i> <i>Proteus morganii</i> <i>Providencia rettgeri</i> <i>Pseudomonas aeruginosa</i> <i>Dermatophilus chelonae</i>	Amikacin Ceftazidime Ticarcillin Enrofloxacin Penicillin G Ampicillin Tetracycline No treatment Immersion in malachite green solution
	Subcutaneous abscesses	<i>Mycobacterium chelonae</i> <i>Mucor</i> <i>Aspergillus</i> <i>Pasteurella testudinis</i> <i>Escherichia coli</i> <i>Providencia</i> <i>Bacteroides</i> <i>Fusobacterium</i>	Fluconazole Amikacin Metronidazole
Oral cavity	Stomatitis	<i>Aeromonas hydrophila</i> <i>Pseudomonas aeruginosa</i> <i>Vibrio</i> spp.	Amikacin Ceftazidime Ticarcillin Enrofloxacin
Respiratory	Pneumonia	<i>Pseudomonas aeruginosa</i> <i>Morganella morganii</i> <i>Serratia marcescens</i> <i>Acinetobacter calcoaceticus</i> <i>Bacteroides</i> <i>Fusobacterium</i> <i>Aspergillus</i> <i>Geotrichum candidum</i> <i>Beauveria</i> <i>Penicillium lilacinum</i> <i>Paecilomyces fumosoroseus</i>	Amikacin Ceftazidime Ticarcillin Enrofloxacin Metronidazole Ketoconazole Itraconazole Fluconazole
	Rhinitis	<i>Pasteurella testudinis</i> <i>Mycoplasma agassizii</i>	Enrofloxacin Tylosin Clarithromycin
Gastrointestinal	Enteritis	<i>Pseudomonas aeruginosa</i> <i>Salmonella</i> spp. <i>Aeromonas hydrophila</i> <i>Chryseobacterium meningosepticum</i>	Trimethoprim/sulfadiazine Ciprofloxacin
	Liver abscesses	<i>Bacteroides</i> <i>Clostridium</i> <i>Fusobacterium</i>	Metronidazole
	Septicemia	<i>Salmonella</i> spp. <i>Aeromonas hydrophila</i> <i>Pseudomonas aeruginosa</i> <i>Chryseobacterium meningosepticum</i>	Amikacin Ceftazidime Ticarcillin Enrofloxacin
Skeletal	Osteomyelitis/arthritis	<i>Pseudomonas</i> <i>Klebsiella</i> <i>Mycobacterium chelonae</i> <i>Nocardia</i> Fungi	Amikacin Ceftazidime No treatment Azithromycin Fluconazole Sulfonamides
Eye and adnexa	Conjunctivitis	<i>Mycoplasma agassizii</i>	Doxycycline Enrofloxacin Clarithromycin
Ear	Otitis interna	<i>Pseudomonas</i> <i>Escherichia coli</i> <i>Proteus</i> <i>Pasteurella testudinis</i> <i>Bacteroides</i> <i>Fusobacterium</i>	Amikacin Ceftazidime Ticarcillin Enrofloxacin Metronidazole

Table 38.2. Antimicrobial drug selection in crocodilian infections.

Site or Type	Diagnosis	Common Infecting Organisms	Suggested Drugs
Oral cavity	Stomatitis	<i>Aeromonas hydrophila</i>	Tetracycline Amikacin Ceftazidime
Skin	Epidermitis/dermatitis	<i>Candida</i>	Nystatin
		<i>Dermatophilus</i>	Procaine penicillin G Ampicillin Oxytetracycline Amikacin Ceftazidime
		<i>Morganella morganii</i> <i>Klebsiella oxytoca</i> <i>Pseudomonas aeruginosa</i> <i>Serratia marcescens</i>	Ketoconazole Itraconazole Fluconazole
Respiratory	Pneumonia	<i>Trichosporon</i>	
		<i>A. hydrophila</i> <i>Citrobacter freundii</i> <i>M. morganii</i> <i>Providencia rettgeri</i> <i>Escherichia coli</i> <i>Salmonella arizonae</i>	Amikacin Ceftazidime Enrofloxacin
		<i>Beauveria</i> <i>Fusarium</i> <i>Mucor</i> <i>Paecilomyces</i>	Ketoconazole Itraconazole Fluconazole
Yolk infection	Omphalitis	<i>Mycoplasma alligatoris</i>	Enrofloxacin Oxytetracycline
		<i>A. hydrophila</i>	Tetracycline Amikacin
Liver	Hepatitis	<i>S. arizonae</i> <i>E. coli</i> <i>A. hydrophila</i> <i>Chlamydia</i>	Amikacin Ceftazidime Enrofloxacin Oxytetracycline
			Enrofloxacin
Eye	Uveitis	<i>A. hydrophila</i>	Amikacin Ceftazidime Tetracycline
			Amikacin
Cardiovascular	Septicemia	<i>S. arizonae</i> <i>A. hydrophila</i>	Ceftazidime Enrofloxacin
			Enrofloxacin
Serosa/joints	Polyserositis/arthritis	<i>Mycoplasma alligatoris</i>	Oxytetracycline

antimicrobial drugs have been reported for exceedingly few. This is not surprising in view of the relatively few researchers interested in PKs of antimicrobials in reptiles and the lack of research support available for such studies. The majority of reptiles are lizards, and the majority of these are too small as

adults to do PK studies. The small numbers of PK studies that have been performed indicate that the half-life of antimicrobial drugs eliminated by excretion (unchanged) is considerably longer in reptiles than in mammalian species. In a study of chloramphenicol elimination, which takes place mainly by he-

Table 38.3. Antimicrobial drug selection for infections in lizards and snakes.

Site or Type	Diagnosis	Common Infecting Organisms	Suggested Drugs
Oral cavity	Stomatitis	<i>Pseudomonas aeruginosa</i> <i>Aeromonas hydrophila</i>	Amikacin Ceftazidime Cefoperazone Piperacillin Enrofloxacin
Skin and subcutis	Abscesses	<i>Proteus</i> <i>Escherichia coli</i> <i>Providencia</i> <i>Pseudomonas</i> <i>Salmonella</i> <i>Serratia</i> <i>Clostridium</i> <i>Fusobacterium</i> <i>Bacteriodes</i>	Amikacin Ceftazidime Piperacillin Enrofloxacin Metronidazole
		<i>Citrobacter</i> <i>Klebsiella</i> <i>Pseudomonas</i> <i>Neisseria</i>	Amikacin Ceftazidime Enrofloxacin
	Mycotic dermatitis	<i>Geotrichum</i> <i>Fusarium</i> <i>Chrysosporium</i>	Ketoconazole Itraconazole Fluconazole
		<i>Pseudomonas aeruginosa</i> <i>Stenotrophomonas maltophilia</i> <i>Salmonella arizonae</i> <i>Providencia rettgeri</i> <i>Aeromonas hydrophila</i> <i>Morganella morganii</i>	Amikacin Ceftazidime Cefoperazone Piperacillin Enrofloxacin Azithromycin
	Pneumonia	<i>S. arizonae</i> <i>P. aeruginosa</i> <i>A. hydrophila</i> <i>E. coli</i>	Trimethoprim/sulfadiazine Ciprofloxacin Metronidazole
Gastrointestinal	Enteritis	<i>P. aeruginosa</i> <i>S. maltophilia</i> <i>M. morganii</i> <i>S. arizonae</i> <i>P. rettgeri</i> <i>Clostridium</i>	Amikacin Ceftazidime Cefoperazone Enrofloxacin Metronidazole
	Hepatitis	<i>Proteus</i> <i>E. coli</i> <i>Pseudomonas</i>	Amikacin Ceftazidime Piperacillin Enrofloxacin
Skeletal	Osteomyelitis	<i>Pseudomonas</i> spp. <i>P. rettgeri</i> <i>Proteus</i> spp. <i>Pseudomonas</i> spp. <i>K. pneumoniae</i>	Topical gentamicin subspectacular space Amikacin Ceftazidime Piperacillin Enrofloxacin
Eye	Subspectacle infections		
	Uveitis		Amikacin Ceftazidime Piperacillin
	Conjunctivitis	<i>Pseudomonas</i> spp.	Amikacin Enrofloxacin
Cardiovascular	Septicemia	<i>A. hydrophila</i> <i>P. aeruginosa</i> <i>S. arizonae</i>	Amikacin Piperacillin Ceftazidime Cefoperazone Enrofloxacin

Table 38.4. Conventional dosage regimens for antimicrobial drugs in reptiles.

Drug	Species	Route	Dose	Dose Interval	References
Amikacin	American alligator	IM	2.25 mg/kg	96 h	Jacobson et al., 1988
	Gopher tortoise	IM	5 mg/kg	48 h	Caligiuri et al., 1990
	Snakes	IM	5 mg/kg; 2.5 mg/kg	1st dose; thereafter 72 h	Mader et al., 1985; Johnson et al., 1997
Ampicillin	Hermann's tortoise	IM	50 mg/kg	12 h	Sporle, 1991
Azithromycin	Ball python	PO	10 mg/kg	2 to 7 days	Coke et al., 2003
Carbenicillin	Snakes	IM	400 mg/kg	24 h	Lawrence, 1984
	Tortoises	IM	400 mg/kg	48 h	Lawrence, 1986
Cefoperazone	Tegu	IM	125 mg/kg	24 h	Speroni, 1989
Ceftazidime	False water cobra	IM	100 mg/kg	96 h	Speroni, 1989
	Snakes	IM, IV	20 mg/kg	72 h	Lawrence, 1984
Chloramphenicol	Loggerhead sea turtle				
	Snakes	SC	50 mg/kg	12-72 h depending on species	Bush, 1978 Clark, 1985
Ciprofloxacin	Snakes	Oral	2.5 mg/kg	48-72 h	Klingenberg and Backner, 1991
Clarithromycin	Desert tortoise	Oral	15 mg/kg	48 to 72 h	Wimsatt et al., 1999
Doxycycline	Tortoise	IM	50 mg/kg; 25 mg/kg	1st dose; 72 h	Sporle, 1991
Enrofloxacin	Box turtle	IM	5 mg/kg	96-120 h	Aucoin, pers. comm.
	Hermann's tortoise	IM	10 mg/kg	24 h	Sporle, 1991
	Gopher tortoise	IM	5 mg/kg	24-48 h	Prezant et al., 1994
	Star tortoise	IM	5 mg/kg	12-24 h	Raphael et al., 1994
	Red-eared slider	IM	5 mg/kg	Not given	James et al., 2003
		PO	10 mg/kg	Not given	
	American alligator	IV	5 mg/kg	36 h	Helmick et al., 2004
	Green iguana	IM	5 mg/kg	24 h	Maxwell et al., 1997
	Savanna monitor	IM	10 mg/kg	5 days	Hungerford et al., 1997
	Burmese python	IM	10 mg/kg	48 h	Young, 1997
Fluconazole	Loggerhead sea turtle	SC	21mg/kg; 10 mg/kg	1st dose; thereafter 5 days	Mallo et al., 2002
Gentamicin	Alligator	IM	1.75 mg/kg	72-96 h	Jacobson et al., 1988
	Aquatic turtles	IM	6-10 mg/kg	48-120 h	Bush et al., 1977
					Raphael et al., 1985
Itraconazole	Snakes	IM	2.5 mg/kg	72 h	Bush et al., 1978
	Kemp's Ridley sea turtle	PO	15 mg/kg	72 h	Manire et al., 2003
			5 mg/kg	24 h	
Ketoconazole	Spiny lizard	PO	23.5 mg/kg	Daily	Gamble et al., 1997
	Tortoise	PO	15-30 mg/kg	24 h	Page et al., 1991
Metronidazole	Green iguana	PO	20 mg/kg	48 h	Kolmstetter et al., 1998
	Yellow rat snake	PO	20 mg/kg	48 h	Kolmstetter et al., 2001
	Red rat snake	PO	20 mg/kg	48 h	Bodri et al., 2006
Nystatin	All species	PO	100,000 IU/kg	24 h	Jacobson, 1980
Oxytetracycline	American alligator	IV	10 mg/kg	5 days	Helmick et al., 2004
	Loggerhead sea turtle	IM	41 mg/kg; 21 mg/kg	1st dose; thereafter 72 h	Harms et al., 2004
		IM	82 mg mg/kg; 42 mg/kg	1st dose; thereafter 72 h	
Piperacillin	Snakes	IM	100 mg/kg	24 h	Hilf et al., 1991
Ticarcillin	Loggerhead sea turtle	IM	50 mg/kg	24 h	Manire et al., 2005
			100 mg/kg	48 h	
Trimethoprim/sulfadiazine	All species	IM	30 mg/kg	1st 2 doses q24h, then q48h	Jacobson, 1987
Tylosin	All species	IM	5 mg/kg	24 h	Jenkins, 1991

patic metabolism, the apparent half-life of chloramphenicol in bull snakes was 5.2 hours, compared with 1.0 and 3.0 hours in mammalian species, with the exception of cats (Bush et al., 1978). Since reptiles constitute a highly diverse collection of species, the validity of interspecies extrapolation of PK data is highly questionable. The dosing interval for chloramphenicol in different species of snakes varied from 12 to 72 hours (Clark et al., 1985). Intra-species differences in dosage may also exist, depending on age and size. For example, a hatchling Burmese python (*Python molurus*) weighing 125 g would probably require a higher dose of antibiotic per kg body weight than an adult python weighing over 100 kg. Metabolic scaling, based on daily minimum energy expenditure rather than live body weight, has been suggested as a method for estimating antimicrobial dosage regimens (Sedgewick et al., 1984), but problems are encountered when this approach is used (Jacobson, 1996).

When selecting an antimicrobial drug, one should consider the immune status of the reptile. Since many ill reptiles, especially those with chronic infections, appear to be immuno-compromised, the use of bactericidal antibiotics is generally preferable (Jacobson, 1987).

Furthermore, since body temperature affects immune system function, it is imperative to maintain the ill reptile under optimum environmental conditions. Ambient temperature has been shown to affect the disposition kinetics of amikacin. Gopher snakes (*Pituophis melanoleucus catenifer*) were given amikacin [5 mg/kg body weight, intramuscularly (IM)] and were housed at ambient temperatures of 25°C and 37°C. When housed at 37°C, the apparent volume of distribution was larger and body clearance of amikacin was higher, while the apparent half-life of the drug did not change significantly (Mader et al., 1985). The mean residence time of amikacin was significantly shorter in gopher tortoises (*Gopherus polyphemus*) acclimated at 30°C (22.67 ± 0.50 hours) than at 20°C (41.83 ± 3.23 hours), and body clearance of the drug in the tortoises acclimated at 30°C was approximately twice that in the tortoises at 20°C (Caligiuri et al., 1990). In addition, oxygen consumption was approximately twice as great in the tortoises at the higher acclimation temperature. In another study in ball pythons (*Python regius*), snakes were acclimated at either 25°C or 37°C and serum concentrations of amikacin were determined following intracardiac and IM administration (3.48

mg/kg) (Johnson et al., 1997). No significant PK differences were found among the snakes housed at these temperatures. It was the view of the authors that the dose administered in this study should produce maximum serum concentrations against most pathogenic bacteria. Thus these findings are not consistent with the few other studies showing temperature effects on blood concentrations of antimicrobials in reptiles.

When being treated with an antimicrobial, maintaining reptiles within their preferred optimum temperature zone is recommended. Snakes affected with respiratory disease have recovered by maintaining them at an elevated environmental temperature without antimicrobial therapy (Ross, 1984). This treatment technique is called *thermotherapy*. Some fungi that infect reptiles grow best at low body temperatures. Raising the body temperature of infected reptiles to the upper limits of their preferred optimum temperature zone may help to limit growth of these fungi.

Size and temperament of a reptile influence antimicrobial drug selection and the method of administration. Most species of reptiles weigh less than 100 g and many lizards are under 30 g as adults. The clinician may be limited to those antibiotics that can easily be diluted to a concentration that can be precisely and safely injected. Microliter syringes are recommended when injecting small (<10 g) reptiles. At the other end of the spectrum are those reptiles that are quite large in size and dangerous to approach. In such cases the clinician may have to choose a drug that can be administered in a relatively small volume, either by hand or via an injection dart. In dangerous reptiles such as venomous snakes, a drug that can be administered every few days is preferred over a drug that must be administered each day. Some reptiles are extremely timid and nervous, and may not be suitable for injection. In such cases the antibiotic must be administered orally, preferably in food if the animal is still feeding. Thus the route of administration will also influence the choice of antibiotic.

The microorganisms that commonly infect various tissues and organs and the drugs recommended for treating these infections are listed in Tables 38.1 to 38.3.

Methods of Administration

In most cases, antimicrobials will be given by injection, either SC or IM. The author generally adminis-

ters oral antimicrobials only in those cases where there is primary infection of the gastrointestinal tract, in those species that do not tolerate injections and hence must be medicated in their feed, or in those disease conditions requiring a drug that is only available in an oral form. Additionally, in farming operations of reptiles such as crocodilians and sea turtles, when large numbers of reptiles are ill and must be treated, it may not be practical to administer drugs by injection. In such cases, oral medication is generally the preferred route of administration.

Several problems exist with oral medication of reptiles. First, very few PK studies have been performed on drugs administered orally to reptiles. Thus, for the vast majority of antimicrobials the dose selected will not be based on science. The gastrointestinal transit time varies greatly among the various groups and species of reptiles, being slowest in the large herbivorous reptiles. The transit time in large tortoises may be as much as 21 days. Even in some carnivorous reptiles, the transit time may be quite prolonged. Thus, in these animals it may be difficult to achieve optimum therapeutic concentrations of antimicrobials in blood following oral administration.

While many oral antimicrobials can be administered in the food of ill reptiles that are feeding, orally medicating reptiles that are not feeding may be difficult. Venomous snakes and large crocodilians are dangerous to handle and manipulate for administration of oral drugs. It may be impossible to extricate the head beyond the shell margins and force open the mouths of many species of turtles and tortoises. The keratinized epidermal hard parts over the mandibles and dentary bones are easily traumatized, and extreme care must be taken in trying to force the mouth open. The giant tortoises are particularly difficult to administer oral drugs. These reptiles must be anesthetized and a pharyngostomy tube inserted for oral medication (Norton et al., 1989). Pharyngostomy tubes are easy to insert in chelonians and are routinely used in injured and ill gopher tortoises and other chelonians admitted to the Zoological Medicine Service at the University of Florida.

As a generalization, snakes are the easiest group of reptiles to orally medicate. The mouth of most snakes is simple to open and because the glottis is in an extremely cranial position, is easily avoided. A lubricated French catheter or nasogastric tube can be passed down the esophagus of the snake with minimal resist-

ance. Catheters that are very rigid should be avoided since they may penetrate the esophageal mucosa. It is important to have the snake relatively straight when passing the catheter. Since the cranial esophagus is extremely thin in most species, the end of the catheter should be round and smooth. While the stomach of most snakes is from one-third to half the distance from the head to the cloaca, it is not necessary to pass a catheter this far. In most situations, passing the catheter halfway between the stomach and oral cavity is satisfactory.

Most of the antibiotics commonly used in reptile medicine are injected IM or SC. The problem with IV administration of antibiotics is that, except in tortoises, certain sized crocodilians, and lizards (Jacobson et al., 1992; Wellehan et al., 2004), peripheral vessels are difficult to catheterize. While blood can be collected from a number of vascular sites in different species of reptiles (Olson et al., 1975; Samour et al., 1984), most of this sampling is "blind" and may not be suitable for repetitive infusions.

With SC and IM drug administration, the author tends to avoid administering drugs that require large volumes per kg of body weight, especially if the drug is irritating to surrounding tissues. For instance, the author has had several snakes develop necrotizing skin lesions following injection of more than 1 cc of enrofloxacin at a single site. A gopher tortoise that received an IM injection of enrofloxacin in a forelimb eventually had to have the limb amputated. The author has seen severe necrosis of pectoralis musculature in sea turtles injected with enrofloxacin. Because of this, the author does not recommend that enrofloxacin be administered by injection to reptiles. Further, since oral studies conducted in several species of reptiles indicate that they can absorb enrofloxacin from the gastrointestinal tract, oral administration is preferred.

Since most species of reptiles have a renal portal system, with blood from the caudal half of the body going to the kidneys before reaching systemic circulation, it has been recommended that SC and IM injections be given in the cranial half of the body. However, there are few studies that have looked at this potential problem critically. In a study in red-eared sliders, *Trachemys scripta elegans*, blood from the caudal region of the body did not necessarily flow through the kidney via the renal portal system (Holz et al., 1997a). Blood draining the caudal portion of the body in the red-eared slider perfuses the liver in addition to, or instead

of, the kidneys. Thus hepatic metabolism also must be considered. When red-eared sliders received either gentamicin (10 mg/kg) or carbenicillin (200 mg/kg) in a forelimb or hindlimb, no significant differences were found in any of the PK parameters in turtles treated with gentamicin, while those that received carbenicillin in a hindlimb had significantly lower blood concentrations for the first 12 hours post-injection than those that received the same dose in a forelimb (Holz et al., 1997b). However, since blood concentrations for both injection sites were still well above the MIC for organisms generally treated with carbenicillin, this difference was not considered clinically significant. Still, the renal portal system varies in development between various groups of reptiles and further work is needed before broad generalizations can be made. This is particularly important when injecting drugs that are potentially nephrotoxic and those that are eliminated primarily through the renal system.

Snakes are the easiest reptiles to inject because of the large dorsal muscle masses associated with the ribs and vertebrae. In lizards, forelimb muscle mass is low and volumes injected must therefore be small. The best site for IM injection in chelonians is the pectoralis musculature. This muscle group is located just medial and caudal to the base of the forelimbs (within the margins of the shell).

A special feature of all snakes with eyes and some lizards is the spectacle (Millichamp et al., 1983). As previously described, the spectacle embryologically represents a fusion of the upper and lower eyelids that has become transparent. It is present as a permanent structure over the cornea and is shed with skin during ecdysis. Infections of the subspectacular space are common in snakes and topical antibiotics do not appear to move across the spectacle. In treating reptiles with such infections, a wedge is removed from the spectacle and the appropriate topical antimicrobials are applied directly onto the globe and within the space.

Metabolic Scaling

Metabolic scaling has become popular in attempting to determine the most appropriate dosage of an antibiotic in a species or size range of animal for which no PK studies have been conducted. Since PK studies have only been performed in a handful of the 7500

species of extant reptiles, most dosages of antibiotics (and other drugs) are given based upon extrapolation from one species to another. In using metabolic scaling, dosages of drugs administered are based upon metabolic size rather than mass. In expressing metabolic rate (P_{met} or minimum energy costs) as a function of body mass (M_b) in kilograms, for most mammals the following allometric equation best describes this relationship (Kleiber, 1961):

$$P_{\text{met}} = 70M_b^{0.75}$$

A similar allometric equation [$\text{MEC} = 10(\text{Kg})^{0.75}$] has been suggested for use in reptiles (Pokras et al., 1992; Sedgwick et al., 1984). In a review of the subject, no single equation was considered appropriate for all reptiles since the mass constant varies from 1 to 5 for snakes and 6 to 10 for lizards; no values for chelonians or crocodilians are available (Jacobson, 1996). In regard to reptiles, the major problem with this approach is a general lack of metabolic data for the majority of extant species. Additionally, there appears to be significant data variability in those groups where scientific studies have been performed. For instance, Bartholomew and Tucker (1964), in looking at metabolic data on lizards ranging in size from 2 g to 4.4 kg, calculated the allometric equation to be $P_{\text{met}} = 6.84M_b^{0.62}$. This is different from findings by Bennet and Dawson (1976) for 24 species of lizards, ranging from 0.01 to 7 kg, for which the equation $P_{\text{met}} = 7.81M_b^{0.83}$ was determined. Further, when one looks at studies with snakes, still different equations can be calculated (Galvao et al., 1965). In determining resting metabolic rates of 34 species of boas and pythons, the mass exponents of different species showed considerable variation (Chappell and Ellis, 1987). The problem with metabolic scaling is that reptiles represent a very heterogeneous group of vertebrates, and because of this, no single equation relating metabolic rate to body mass can be developed for calculating antibiotic dosages. Differences in body temperature, season, reproductive status, nutrition, and overall physiology are just a few of the variables which may ultimately influence metabolic rates, making application of a single equation impossible. While at first glance metabolic scaling may appear better than extrapolation, developing a single equation for all reptiles may not be valid. Metabolic scaling will be most useful when calculating doses in a species for which a specific equation has been determined.

Dosage Regimens

Suggested dosage regimens for antimicrobial drugs in various species of reptiles are presented in Table 38.4.

Bibliography

- Aucoin D. 1991. N Carolina State Univ Coll Vet Med, Raleigh, NC (personal communication).
- Austwick PKC, Keymer IF. 1981. Fungi and actinomycetes. In: Cooper JE, Jackson OF, eds. *Diseases of the Reptilia*. V1. London: Academic Press.
- Barrett S, et al. 1994. A new species of *Neisseria* from iguanid lizards, *Neisseria iguanae* sp. nov. *Lett Appl Micro* 18:200.
- Bartholomew GA, Tucker VA. 1964. Size, body temperature, thermal conductance, oxygen consumption, and heart rate in Australian varanid lizards. *Physiol Zoo* 37:341.
- Bennet AF, Dawson WR. 1976. Metabolism. In: Gans C, Dawson WR, eds. *Biology of the Reptilia, Physiology A*. V5. New York: Academic Press.
- Bertelsen ME, et al. 2005. Fatal cutaneous mycosis in tentacled snakes (*Erpeton tentaculatum*) caused by the *Chrysosporium* anamorph of *Nannizziopsis vriesii*. *J Zoo Wildl Med* 36:82.
- Bodetti, et al. 2002. Molecular evidence to support the expansion of the host range of *Chlamydia pneumoniae* to include reptiles as well as humans, horses, koalas and amphibians. *Syst Appl Microbiol* 25:146.
- Bodri MS, et al. 2006. Pharmacokinetics of metronidazole administered as a single oral bolus to red rat snakes, *Elaphe guttata*. *J Herpetol Med Surg* 16:15.
- Brown DR, et al. 2001. *Mycoplasma alligatoris* sp. nov., from American alligators. *Int J Syst Evol Microbiol*. 51:419.
- Brown MB, et al. 1995. *Mycoplasma agassizii* causes upper respiratory tract disease in the desert tortoise. *Inf Immun* 62:4580.
- Brown MB, et al. 1999. Upper respiratory tract disease in the gopher tortoise is caused by *Mycoplasma agassizii*. *J Clin Microbiol* 37:2262.
- Bush M, et al. 1976. Biological half-life of gentamicin in gopher snakes. *Am J Vet Res* 39:171.
- Bush M, et al. 1977. Preliminary study of gentamicin in turtles. *Proc Am Assoc Zoo Vet*, p71.
- Bush M, et al. 1978. Preliminary study of antibiotics in snakes. *Proc Amer Assoc Zoo Vet*, p50.
- Caligiuri RL, et al. 1990. The effects of ambient temperature on amikacin pharmacokinetics in gopher tortoises. *J Vet Pharmacol Ther* 13:287.
- Chappell MA, Ellis TM. 1987. Resting metabolic rates in boid snakes: allometric relationships and temperature effects. *J Comp Physiol B*. 157:227.
- Clark CH, et al. 1985. Plasma concentrations of chloramphenicol in snakes. *Am J Vet Res* 46:2654.
- Clark HF, Lunger PD. 1981. Viruses. In: Cooper JE, Jackson OF, eds. *Diseases of the Reptilia*. V1, p135. London: Academic Press.
- Clippinger TL, et al. 2000. Morbidity and mortality associated with a new *Mycoplasma* species from captive American alligators (*Alligator mississippiensis*). *J Zoo Wildl Med* 31:303.
- Coke RL, et al. 2003. Pharmacokinetics and tissue concentrations of azithromycin in ball pythons (*Python regius*). *Am J Vet Res* 64:225.
- Cooper JE. 1981. Bacteria. In: Cooper JE, Jackson OF, eds. *Diseases of the Reptilia*. V1. London: Academic Press.
- Galvao PE, et al. 1965. Heat production of tropical snakes in relation to body weight and body surface. *Am J Phys* 209:501-506.
- Gamble KC, et al. 1997. Itraconazole plasma and tissue concentrations in the spiny lizard (*Sceloporus* sp.) following once daily dosing. *J Z Wildl Med* 28:89.
- Hanel R, et al. 1999. Isolation of *Clostridium* spp. from the blood of captive lizards: real or pseudobacteremia. *Bull Assoc Reptil Amphib Vet* 9:4.
- Harms CA, et al. 2004. Pharmacokinetics of oxytetracycline in loggerhead sea turtles (*Caretta caretta*) after single intravenous and intramuscular injections. *J Zoo Wildl Med* 35:477.
- Helmick KE, et al. 2004. Kinetic disposition of enrofloxacin following single-dose oral and intravenous administration in the American alligator (*Alligator mississippiensis*). *J Zoo Wildl Med* 35:361.
- Helmick KE, et al. 2004. Pharmacokinetic disposition of a long acting oxytetracycline formulation following single-dose intravenous and intramuscular administrations in the American alligator (*Alligator mississippiensis*). *J Zoo Wildl Med* 35:367.
- Hilf M, et al. 1989. Pharmacokinetics of gentamicin and piperacillin in blood pythons: new dosing regimen. *Proc 13th Inter Symp Prop Husband Rept*, p87.
- Hilf M, et al. 1991. Pharmacokinetics of piperacillin in blood pythons (*Python curtus*) and in vitro evaluation of efficacy against aerobic gram-negative bacteria. *J Zoo Wildl Med* 22:199.
- Holz P, et al. 1997a. The anatomy and perfusion of the renal portal system in the red-eared slider (*Trachemys scripta elegans*). *J Zoo Wildl Med* 28:378.
- Holz P, Barker IK, Burger JP, et al. 1997b. The effect of the renal portal system on pharmacokinetic parameters in the red-eared slider (*Trachemys scripta elegans*). *J Zoo Wildl Med* 28: 386.
- Homer BL, et al. 1994. Chlamydiosis in green sea turtles. 1994. *Vet Pathol* 31:1.
- Hungerford C, et al. 1997. Pharmacokinetics of enrofloxacin after oral and intramuscular administration in savanna monitors (*Varanus exanthematicus*). *Proc Amer Assoc Zoo Vet*, p89.
- Hunter RP, et al. 2003. Azithromycin metabolite identification in plasma, bile, and tissues of the ball python (*Python regius*). *J Vet Pharmacol Ther* 26:117.
- Jacobson ER. 1980. Infectious diseases of reptiles. In Kirk RW, ed. *Current Therapy*. V7. Philadelphia: WB Saunders.
- Jacobson ER. 1987. Reptiles. In Harkness J, ed. *Veterinary Clinics of North America*. Philadelphia: WB Saunders.

- Jacobson ER. 1992. Laboratory investigations. In Lawton MPC, Cooper JE, eds. *Manual of Reptiles*. Gloucestershire: Brit Sm An Vet Assn.
- Jacobson ER. 1996. Metabolic scaling of antibiotics in reptiles: basis and limitations. *Zoo Biol* 15:329.
- Jacobson ER. 1999. Use of antimicrobial drugs in reptiles. In: Fowler ME, Miller RE, eds. *Zoo and Wild Animal Medicine, Current Therapy 4*. Philadelphia: WB Saunders.
- Jacobson ER, Telford SR. 1990. *Chlamydia* and poxvirus infection of monocytes in a flap-necked chameleon. *J Wildl Dis* 26:572.
- Jacobson ER, et al. 1988. Serum concentration and disposition kinetics of gentamicin and amikacin in juvenile American alligators. *J Zoo Anim Med* 19:188.
- Jacobson ER, et al. 1989. Chlamydial infection in puff adders, *Bitis arietans*. *J Zoo Wildl Med* 20:364.
- Jacobson ER, et al. 1991. Chronic upper respiratory tract disease of free-ranging desert tortoises (*Xerobates agassizii*). *J Wildl Dis* 27:296.
- Jacobson ER, et al. 1992. Techniques for sampling and handling blood for hematologic and plasma biochemical determinations in the desert tortoise (*Xerobates agassizii*). *Copeia* 1:237.
- Jacobson E, et al. 2002. Immunohistochemical staining of chlamydial antigen in emerald tree boas (*Corallus caninus*). *J Vet Diag Investig* 14:487.
- Jacobson ER, et al. 2005. Plasma concentrations of enrofloxacin after single dose oral administration in loggerhead sea turtles (*Caretta caretta*). *J Zoo Wildl Med* 36:628.
- Jacobson ER, Heard D, Andersen A. Identification of *Chlamydiophila pneumoniae* in emerald tree boas, *Corallus caninus*. *J Vet Diag Investig* 16:153.
- James SB, et al. 2003. Comparison of injectable versus oral enrofloxacin pharmacokinetics in red-eared slider turtles, *Trachemys scripta elegans*. *J Herp Med Surg* 13:5.
- Jenkins JR. 1991. Medical management of reptiles. *Compend Contin Educ Pract Vet* 13:980.
- Johnson JH, et al. 1997. Amikacin pharmacokinetics and the effects of ambient temperature on the dosage regimen in ball pythons (*Python regius*). *J Zoo Wildl Med* 28:80.
- Kirchoff H, et al. 1997. *Mycoplasma crocodyli* sp. nov., a new species from crocodiles. *Int J Syst Bacteriol* 47:742.
- Kleiber, M. 1961. *The Fire of Life: An Introduction to Animal Energetics*. New York: Wiley.
- Klingenberg RJ. 1996. Therapeutics. In: Mader DR, ed. *Reptile Medicine and Surgery*. Philadelphia: WB Saunders.
- Klingenberg RJ, Backner B. 1991. The use of ciprofloxacin, a new antibiotic, in snakes. *Proc 15th Inter Symp Captive Prop Husb Rept Amphib*, p127.
- Kolmstetter CM, et al. 1998. Pharmacokinetics of metronidazole in the green iguana, *Iguana iguana*. *Bull Amer Assoc Reptil Amphib Vet* 8:4.
- Kolmstetter CM, et al. 2001. Pharmacokinetics of metronidazole in the yellow ratsnake (*Elaphe obsoleta quadrivittata*). *J Herp Med Surg* 11:4.
- Lawrence K. 1984. Preliminary study on the use of cefazidime, a broad-spectrum cephalosporin antibiotic, in snakes. *Res Vet Sci* 36:16.
- Lawrence K, et al. 1984. A preliminary study on the use of carbenicillin in snakes. *J Vet Pharmacol Ther* 7:119.
- Lawrence K, et al. 1986. Use of carbenicillin in two species of tortoise (*Testudo graeca* and *T. hermanni*). *Res Vet Sci* 40:413.
- Mader DR, et al. 1985. Effects of ambient temperature on the half-life and dosage regimen of amikacin in the gopher snake. *J Am Vet Med Assoc* 187:1134.
- Mallo KM, et al. 2002. Pharmacokinetics of fluconazole in loggerhead sea turtles (*Caretta caretta*) after single intravenous and subcutaneous injections, and multiple subcutaneous injections. *J Zoo Wildl Med* 33:29.
- Manire CA, et al. 2003. Steady-state plasma concentrations of itraconazole after oral administration in Kemp's Ridley sea turtles, *Lepidochelys kempi*. *J Zoo Wildl Med* 34:171.
- Manire CA, et al. 2005. Pharmacokinetics of ticarcillin in the loggerhead sea turtle (*Caretta caretta*) after single intravenous and intramuscular injections. *J Zoo Wildl Med* 36:44.
- Maxwell LK, and Jacobson ER. 1997. Preliminary single-dose pharmacokinetics of enrofloxacin after oral and intramuscular administration in green iguanas (*Iguana iguana*). *Proc Amer Assoc Zoo Vet*, p 25.
- Migaki G, et al. 1984. Fungal diseases of reptiles. In: Hoff GL, et al, eds. *Diseases of Amphibians and Reptiles*. New York: Plenum Press.
- Millichamp N, et al. 1983. Diseases of the eye and ocular adnexae in reptiles. *J Am Vet Med Assoc* 183:1205.
- Mohan K, et al. 1995. *Mycoplasma*-associated polyarthritis in farmed crocodiles (*Crocodylus niloticus*) in Zimbabwe. *Onderstepoort J Vet Res* 62:45.
- Montali R. 1988. Comparative pathology of inflammation in the higher vertebrates (reptiles, birds and mammals). *J Comp Pathol* 99:1.
- Norton TM, et al. 1989. Medical management of a Galapagos tortoise (*Geochelone elephantopus*) with hypothyroidism. *J Zoo Wildl Med* 20:212.
- Olson GA, et al. 1975. Techniques for blood collection and intravascular infusions of reptiles. *Lab Anim Sci* 25:783.
- Page CD, et al. 1991. Multiple dose pharmacokinetics of ketoconazole administered to gopher tortoises (*Gopherus polyphemus*). *J Zoo Wildl Med* 22:191.
- Paré JA, et al. 1997. Cutaneous mycoses in chameleons caused by the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (Apinis) Currah. *J Zoo Wildl Med* 28:443.
- Paré JA, et al. 2003. Cutaneous mycobiota of captive squamate reptiles with notes on the scarcity of *Chrysosporium* anamorph of *Nannizziopsis vriesii*. *J Herp Med Surg* 13:10.
- Plowman CA, et al. 1987. Septicemia and chronic abscesses in iguanas (*Cyclura cornuta* and *Iguana iguana*) associated with a *Neisseria* species. *J Zoo Anim Med* 18:86.
- Pokras MA, et al. 1992. Therapeutics. In: Beynon PH, et al., eds. *Manual of Reptiles*. Gloucestershire: Brit Sm An Vet Assn.
- Prezant RM, et al. 1994. Plasma concentrations and disposition kinetics of enrofloxacin in gopher tortoises (*Gopherus polyphemus*). *J Zoo Wildl Med* 25:82.
- Raphael B, et al. 1985. Plasma concentrations of gentamicin in turtles. *J Zoo Anim Med* 16:138.

- Raphael BL, et al. 1994. Pharmacokinetics of enrofloxacin after a single intramuscular injection in Indian star tortoises (*Geochelone elegans*). J Zoo Wildl Med 25:88.
- Riff LJ, Jackson GG. 1972. Laboratory and clinical conditions for gentamicin inactivation by carbenicillin. Arch Intern Med 130:887.
- Ross RA. 1984. The Bacterial Diseases of Reptiles. Stanford, CA: Inst Herpetological Res.
- Samour HJ, et al. 1984. Blood sampling techniques in reptiles. Vet Rec 114:472.
- Sedgewick CJ, et al. 1984. Scaling antimicrobial drug dosage regimens to minimum energy cost rather than body weight. Proc Am Assoc Zoo Vet, p 15.
- Shotts EB, et al. 1972. *Aeromonas* induced death among fish and reptiles in an eutrophic inland lake. J Am Vet Med Assoc 161:603.
- Soldati G, et al. 2004. Detection of mycobacteria and chlamydiae in granulomatous inflammation of reptiles: a retrospective study. Vet Pathol 41:388.
- Speroni JA, et al. 1989. Farmacocinética de ceforazona tras su administración intramuscular a *Hydronastes gigas* (Ophidia, Colubridae) mantenidas a 24°C. <http://www.ceih.org/CefoHydroFiles/CefoHydro.php>.
- Speroni JA, et al. 1989. Farmacocinética de ceforazona tras su administración intramuscular a *Tupinambis teguixin* (Sauria, Teiidae). <http://www.ceih.org/CefoHydroFiles/CefoTupi.php>.
- Sporle H, et al. 1991. Blood levels of some anti-infectives in the Hermann's tortoise (*Testudo hermanni*). 4th Int Colloq Pathol Med Reptiles Amphibians, p120.
- Stamper MA, et al. 1999. Pharmacokinetics of ceftazidime in loggerhead sea turtles (*Caretta caretta*) after single intravenous and intramuscular injections. J Zoo Wildl Med 30:32.
- Stamper MA, et al. 2003. Pharmacokinetics of florfenicol in loggerhead sea turtles (*Caretta caretta*) after single intravenous and intramuscular injections. J Zoo Wildl Med 34:3.
- Wellehan JFX, et al. 2004. Coccygeal vascular catheterization in lizards and crocodilians. J Herp Med Surg 14:26.
- Wimsatt J, et al. 1999. Clarithromycin pharmacokinetics in the desert tortoise (*Gopherus agassizii*). J Zoo Wildl Med 30:36.
- Young LA, et al. 1997. Disposition of enrofloxacin and its metabolite ciprofloxacin after IM injection in juvenile Burmese pythons (*Python molurus bivittatus*) J Zoo Wildl Med 28:71.

Antimicrobial Drug Use in Aquaculture

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Antimicrobial use in aquaculture has become rather controversial. Recently, public health agencies have raised concerns worldwide about the impact of antimicrobial use in aquaculture on environmental bacteria and, potentially, on human pathogens (OATA, 1999; Benbrook, 2002; MacMillan, 2003; FAO, 2003; MacMillan et al., 2003). Nevertheless, fish are vertebrates that should receive humane care, including treatment with antimicrobials where appropriate. Prescribing the appropriate antimicrobial, however, can be problematic for several reasons. First, historically, veterinary practitioners have received minimal training in fish medicine. Second, information regarding pharmacokinetics, antimicrobial dosages, and the effects of antimicrobial drugs in fish is not readily available. Third, especially in the United States, only a few antimicrobials are approved for use in food or ornamental fish (Joint Subcommittee on Aquaculture, 2004; US FDA, 2005). Finally, standardized antimicrobial susceptibility testing methods have not been developed for aquatic pathogens, which prefer to grow at temperatures lower than terrestrial pathogens. Thus the practitioner must often interpret test results generated by a laboratory that has no standardized testing protocol as a reference, and then must prescribe a drug (either labeled or as an extra-label prescription) for which there is little or no literature detailing pharmacokinetic data in the fish species being treated.

Over the last decade, however, researchers and fish health practitioners worldwide have made considerable progress toward obtaining and making available information about therapeutic treatments in aquaculture. A number of textbooks that include formularies for doses and treatment regimens for fish have been published (Stoskopf, 1993; Noga, 1996; Carpenter, 2005). In addition, an extensive review of literature

pertaining to fish pharmacokinetics (Phish-Pharm) has been published as a database, available free in a web-based journal (Reimschuessel et al., 2005). The information in these texts and in the database are, however, skewed toward the species and drugs that are most commonly used. Still missing to a large degree is information on the many exotic and ornamental fish species, as well as the “niche” food-fish species.

Treating fish with antimicrobials is somewhat more complex than treating terrestrial animals, but the basic principles for antimicrobial use are really the same (Chapter 6). Five main aspects must be considered: (1) choice of an appropriate drug at an effective dose, (2) avoidance of toxicity in the animal, (3) insurance of safety for humans administering the antimicrobial or consuming the fish, (4) consideration of non-target species and environmental effects, and (5) awareness of legal restrictions.

Choosing the Appropriate Drug

The Pathogen

Picking an effective drug requires that the clinician make an accurate diagnosis (Chapter 6). A definitive diagnosis requires isolation and identification of the causative organism, preferably from three to five infected fish (Hawke and Thune, 1992; OIE, 2003; American Fisheries Society, 2005). In aquaculture, especially in intensive rearing facilities, timely institution of treatment is often critical. Therefore, many practitioners rely on clinical signs and past experience when faced with a population of moribund fish. It is, however, prudent to take multiple samples for culture prior to administering the antibiotic empirically. This allows confirmation later that the appropriate drug

was used or, if necessary, subsequent change of the treatment based on the organism isolated and its antimicrobial susceptibility profile. Since antimicrobial susceptibility changes with antibiotic use, it is important for the veterinarian to monitor the isolates from their aquaculture clients so that future outbreaks are not treated empirically with the incorrect drug.

Evaluating the antimicrobial susceptibility of aquatic bacterial isolates has been problematic in the past, since there were no standardized methods for testing organisms which grow at lower temperatures than most terrestrial animal pathogens. Recently CLSI (Clinical Laboratory Standards Institute, formerly NCCLS) has published proposed guidelines (CLSI, 2006a, 2006b) for testing aquatic bacteria at different temperatures. Specific methods that should be used for testing aquatic bacteria may be found in these documents. It is essential that these standardized methods be used to ensure reliable results and allow comparisons between laboratories (Chapter 2).

The Host

Choosing the right drug depends in part on such factors as age, size, and housing of the animal. Treatment options will be different for animals that are held in net pens at sea, as opposed to those held in an indoor facility or aquarium. A treatment must also be feasible: An appropriate treatment route for aquarium fish or selected brood-stock individuals may be cost- or labor-prohibitive in commercial aquaculture ventures. The stress associated with treatments must be balanced with the need for and expected benefits of treatment.

Drug dosage regimens also are host-dependent. Fish species reared in warm water may absorb, metabolize and excrete drugs at a different rate (often faster) than those in cold water. The salinity of the holding water also affects drug kinetics. Fish kept in saltwater drink the water while freshwater fish do not. Thus, antimicrobials in the gastrointestinal tract of fish species held in saltwater may bind cations that can reduce their uptake. This is especially true for antimicrobials, such as the tetracyclines, that have low bioavailability even in freshwater fish (Elema et al., 1996). Uptake of oral difloxacin by Atlantic salmon is ten-fold less in saltwater than freshwater (100 vs. 1000 ng/ml in plasma) (Elston et al., 1994)). The elimination rate of oxolinic acid (oral or injected) is also faster in rainbow trout held in saltwater than freshwater (Ishida, 1992). It is therefore important to obtain information on the pharmacoki-

netic parameters of the drugs in the host species. This is, of course, easier said than done. An extensive review of the literature has been incorporated into the Phish-Pharm Database (Reimschuessel et al., 2005), which makes much of the published data rapidly searchable. However, even with such a tool, there are many species and drugs for which there are no published studies. The veterinarian is thus often in the position of making a best guess based on data from other species held, hopefully, under similar conditions. Half-lives of drugs in fish are highly dependent on the dosage regimen, the route, and temperature. Therefore, these parameters are included in the Phish-Pharm Database and should be considered when administering antimicrobials to fish.

The Dosage

Table 39.1 shows drug dosages that have been reported for fish. These dosages are compiled from a number of formularies (Stoskopf, 1993; Noga, 1996; Carpenter, 2005) and research reports. It is important to realize that the dosages listed in Table 39.1 may not have been shown to be safe or effective in all fish species. The table also lists the interval that was reported in the original citation, but it is important to remember that successful therapy often depends on maintaining adequate blood levels over a course of seven to ten days. In some cases, only the dose used for experimental purposes is listed. It is advisable to consider the half-life of the drug in that species when determining the length and frequency of treatments. In a few species (the agglomerular fish in particular), half-lives of drugs excreted by the kidney are quite prolonged (Jones et al., 1997) and must be considered when treating these animals.

Temperature is a very important factor in deciding the dose and treatment intervals. Again, knowledge of drug half-lives calculated from exposures at different temperatures can help the clinician choose intervals that will maximize chances for successful therapy. The half-lives listed in the Phish-Pharm Database (Reimschuessel et al., 2005) may help with such decisions. Temperature as a variable in pharmacokinetics has been reported for a number of drugs. For example, Bowser et al. (1992) examined the half-life of enrofloxacin in rainbow trout at 10 vs. 15°C. The half-life at 10°C was 30 hours, whereas at 15°C the half-life was 56 hours. In a study of flumequine in rainbow trout, however, researchers found that half-lives decreased with rising temperature: 3°C–569 hours,

Table 39.1. Antimicrobial dosages used in fish.

Drug	Dosage	Interval	Route	Comments
Amikacin	5 mg/kg	q 12 h	IM	
	5 mg/kg	q 72h x 3	IM	
Amoxicillin	25 mg/kg	q 12h	PO	Rarely used due to few Gram-positive pathogens.
	40-80 mg/kg	q 24h 10 d	PO	
Ampicillin	10 mg/kg	q 24 h	IM	Sharks
	10 mg/kg	q 12 h 7-10 d	PO	Sharks
	50-80 mg/kg	q 24 h 10 d	PO	
Aztreonam	100 mg/kg	q 48 h 7d	IM/IP	Used by koi hobbyists.
Ceftazidime	22 mg/kg	q 72-96 h x3-5	IM/IP	
Chloramine-T	20 mg/L	1 h 4 d	Bath	
	2.5-20 mg/L	Flush (various)	Bath	Disinfectant control bacterial gill disease and parasites.
	5-10 mg/L	1 h	Bath	
Ciprofloxacin ^a	15 mg/kg	Single dose	IM/IV	Dose used for determining PK.
Difloxacin ^a	10 mg/kg	Single dose	PO	Dose used for determining PK.
Dihydrostreptomycin	0.125 mg	Single dose	IM/IV	Dose used for determining PK.
	10 mg	Single dose	PO	Dose used for determining PK.
	10 mg/kg	q 24 h	IM	Sharks
Enrofloxacin ^a	2.5-5.0 mg/L	5 h q 24 h x 5-7 d	Bath	
	5-10 mg/kg	q 24 h 10 d	PO	
	5-10 mg/kg	q 48 h 10 d	IM/IP	
Erythromycin	10-20 mg/kg	Single dose	IP	For BKD before spawning.
	50-100 mg/kg	q 24 h 10-21 d	PO	
	2 mg/L	1 h	Bath	For BKD in eggs.
Florfenicol	5-20 mg/kg	q 24 h 10 d	PO	Salmon
	10 mg/kg	q 24 h x10 d	PO	Dose approved by USDA for select species.
	40-50 mg/kg	q 12-24 h	PO, IM, IP	Red Pacu
Flumequine ^a	50-100 mg/L	3 h	Bath	Increase dose in saltwater.
	10 mg/kg	q 24 h 10 d	PO	
	20 mg/kg	q 24 h 10 d	PO	
	30 mg/kg		IM/IP	IP (and IM) dosages remain at effective levels for 10d.
Fumagillin	30-60 mg/kg		PO	Dose used for determining PK.
	3-6 mg/kg		IV	Dose used for determining PK.
Gentamicin	3 mg/kg	q 72 h	IM	Very nephrotoxic to aglomerular fish. Bath exposure does not achieve blood levels.
	6 mg/kg	Each week	IM	Sharks
Kanamycin	50-100 mg/L	q 72 h 5 h x 3	Bath	Nephrotoxic some species. Change water 50% between treatments.
	50 mg/kg	q 24 h	Po	Nephrotoxic some species.
	10-20 mg/kg	q 24 h	PO	Sharks
Kanamycin	20 mg/kg	q 72h x 5	IP	Nephrotoxic some species.
Lincomycin	40 mg/kg	q 24 h	PO	Japan
Miloxacin	60 mg/kg	q 24 h 6 d	PO	Japan
Nalidixic acid	13 mg/L	1-4 h	Bath	
	20 mg/kg	q 24 h	PO, IM, IV	Other doses used for PK studies.
Neomycin	66 mg/L	q 3 d x 3	Bath	Toxic to nitrifying bacteria in filter.
	20 mg/kg	Single dose	PO	Sharks to prevent bloat; poorly absorbed from gut.
Oxolinic acid	25 mg/L	0.25 h q 12 h x3 d	Bath	
	1 mg/L	24 h	Bath	
	10 mg/kg	q 24 h x 10d	PO	Freshwater species.
	25-75 mg/kg	q 24 h x 10 d	PO	Saltwater species.
Oxytetracycline	10-50 mg/L	1 hr	Bath	For superficial infections.
	20-50 mg/L	5-24 h q 24 h x 5-6 d	Bath	Change 50-75% water between treatments.
	55-83 mg/kg	q 24 h x 10d	PO	Dose approved by USDA for select species.

(continued)

Table 39.1. Antimicrobial dosages used in fish.

Drug	Dosage	Interval	Route	Comments
	25-50 mg/kg	q 24 h x 5-7 d	IM/IP	Produces high levels for several days when given IM.
	3 mg/kg	q 24 h	IV	Red pacu
Piromidic acid	10 mg/kg	q 24 h 5-10 d	PO	Japan
Sarafloxacin ^a	10-14 mg/kg	q 24 h x 10 d	PO	
Sulfadiazine-trimethoprim	30-50 mg/kg	q 24 h x 7-10 d	PO	
	125 mg/kg		IP	
Sulfadimethoxine-orometoprim	50 mg/kg	q 24 h x 5d	PO	Dose approved by USDA for select species.
Sulfamerazine	220 mg/kg	q 24 h x 14 d	PO	Dose approved by USDA for select species.
	200 mg/kg	q 24 h x 10 d	PO	
Sulfamethoxazole-trimethoprim	20 mg/L	5-12 h q 24 h x 5-7 d	Bath	Change 50-75% water between treatments.
	30 mg/kg	q 24 h x 10-14 d	PO	
Thiamphenicol	20 mg/L	1 h	Bath	
	50 mg/kg	q 24 h x 7-10	PO	Japan

^aExtra label use of fluoroquinolones in food animals is prohibited by US FDA.

7°C–300 hours, and 13°C–137 hours (Sohlberg et al., 1990, 1994). Bjorklund and Bylund (1990) found longer half-lives at 5 and 10 vs. 16°C in rainbow trout fed oxytetracycline, whereas Chen et al. (2004) found minimal differences in a number of fish species held at different temperatures. There is considerable pharmacokinetic data in the Fish-Pharm Database, and Table 39.2 summarizes half-life ranges of antimicrobial drugs in different fish species which have been extracted from the database.

The Treatment Route

Waterborne Treatments

Waterborne antimicrobial treatments will vary depending on the animal and holding conditions. Treating fish by applying the drug to the water avoids stressing the fish by handling. Three main methods are employed: (1) baths (and dips), in which the drug is added to a holding system; (2) flushes, used in flow-through systems, adding the entire dose in a short period (1–2 minutes) then allowing the system to flow, thereby diluting the dose; and (3) constant flow, also used in flow-through systems by continuously pumping in a stock solution with a chemical dosimeter.

Disadvantages of waterborne treatments include expense, waste, and potential environmental contamination. Biological filters may also be compromised due to killing the filter bacteria. A rapid rise in ammonia has been seen using therapeutic concentrations of erythromycin in a catfish recirculating system, but sulfamerazine, oxytetracycline, nifurpirinol, and chloramphenicol did not affect the filter function (Treves-Brown, 2000).

ramphenicol did not affect the filter function (Treves-Brown, 2000).

It is also important to consider the ability of a drug to be absorbed from the water. Lipophilic compounds under a molecular weight of 100 will be more likely to diffuse across the gills. Antimicrobials which are absorbed from the water include chloramines, dihydrostreptomycin, enrofloxacin, erythromycin, flumequine, furfurinol, kanamycin, oxolinic acid, oxytetracycline, nifurpirinol, sulfadimethoxine, sulfadimidine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfisomidine and trimethoprim. Antimicrobials that are absorbed poorly or not at all include chloramphenicol and gentamicin (Treves-Brown, 2000; Reimschuessel et al., 2005).

Dipping treatments are a shorter and more controlled method of administering bath treatments. The advantages of this type of treatment are reduced waste (thus reduced expense) and less environmental contamination. The disadvantage of this type of approach is the increased stress to the animals through handling. Therefore most dip treatments are done when fish are small or in pet/aquarium cases, but commercial aquaculture producers have used tarpaulins to contain the drug (Struhsaker, 1973; Vavarigos, 2003). Localized topical treatment, often under light anesthesia, has been recommended for small external lesions in pet fish (Stoskopf, 1993; Noga, 1996).

Some studies have used either hyperosmotic infiltration (first high osmolarity, >1200 mOsm/L, then lower osmolarity containing the drug) or ultrasound

Table 39.2. Half-lives and dosages of antibiotics in fish.

Drug	Species	T ^{1/2} (hr)	Dosage	Route	°C
Amoxicillin	Atlantic salmon	120	12.5 mg/kg sd*	IM	13
	Atlantic salmon, seabream	14-72	40-80 mg/kg sd	IV/PO	16-22
Chloramphenicol	Carp	48-72	40 mg/kg sd	IP	9
Ciprofloxacin	Carp, rainbow trout, African catfish	11-15	15 mg/kg sd	IM/IV	12-25
Diffloxacin	Atlantic salmon	16	10 mg/kg sd	PO	11
Enrofloxacin	Atlantic salmon, red pacu, rainbow trout, sea bass, seabream	24-105	5-10 mg/kg sd	IM/IV/PO	10-26
Erythromycin	Chinook salmon	120	0.1 g/kg 21d	PO	10
Florfenicol	Atlantic salmon	12-30	10 mg/kg sd	IV/PO	10-11
	Cod	39-43	10 mg/kg sd	IV/PO	8
Flumequine	Eel	255	9 mg/kg sd	IM	23
	Atlantic halibut, brown trout, corksing wrasse, Atlantic halibut, Atlantic salmon, cod, goldsinny wrasse, sea bass, seabream, turbot	21-96	5-25 mg/kg sd	IP/IV/PO	5-25
	Eel	208-314	10 mg/kg sd	IV/PO	23
	Rainbow trout	285-736	5 mg/kg sd	IV/PO	13 vs 3
Furazolidone	Channel catfish	1-24	1 mg/kg sd	IV/PO	24
Gentamicin	Channel catfish, brown shark, goldfish	12-54	1-3.5 mg/kg sd	IC/IM	20-25
	Toadfish	602	3.5 mg/kg sd	IM	19
Miloxacin	Eel	35	30-60 mg/kg sd	IV/PO	27
Nalidixic acid	Rainbow trout, amago salmon	21-46	5-40 mg/kg sd	IV/PO	14-15
Nifurstyrenate	Yellowtail	2	100 mg/kg sd	PO	23
Ormetoprim	Atlantic salmon, channel catfish, rainbow trout, hybrid striped bass	4-25	4-50 mg/kg sd	IV/PO	10-28
Oxolinic acid	Atlantic salmon, corksing wrasse, channel catfish, cod, rainbow trout, red seabream, sea bass	15-87	4-20 mg/kg sd	IP/IV	8-24
	Atlantic salmon, cod, rainbow trout	82-146	25-75 mg/kg sd	PO	5-8
	Atlantic salmon, gilthead seabream, rainbow trout, sharpsnout seabream, turbot	13-48	10-40 mg/kg up to 10d	PO	9-19
Oxytetracycline	African catfish, carp, rainbow trout, red pacu, sockeye salmon	63-95	5-60 mg/kg sd	IM	12-25
	African catfish, Atlantic salmon, ayu, carp, Chinook salmon, eel, rainbow trout, red pacu, sea bass, seabream, sharpsnout seabream	6-167	5-60 mg/kg sd	IV	8-25
	Arctic charr	266-327	10-20 mg/kg sd	IV	6
	Atlantic salmon, ayu, black seabream, carp, channel catfish, eel, perch, rainbow trout, sea bass, seabream, hybrid striped bass, summer flounder, walleye	43-268	10-100 mg/kg up to 10d	PO	7-27
	Arctic charr, sockeye salmon, Chinook salmon	428-578	10-100 mg/kg sd	PO	6-11
Piromidic acid	Eel, goldfish	24	5 mg/kg sd	PO	26
Sarafloxacin	Atlantic salmon, cod	12-45	10-15 mg/kg sd	IV/PO	8-24
Streptozotocin	Toadfish	24	50 uCi	IV	
Sulfachlorpyridazine	Channel catfish	4-5	60 mg/kg sd	IC/PO	22
Sulfadiazine	Atlantic salmon, carp, rainbow trout	26-96	25-200 mg/kg sd	IV/PO	8-24
Sulfadimethoxine	Atlantic salmon, channel catfish, rainbow trout, hybrid striped bass	1-48	25-200 mg/kg sd	IV/PO	10-20
Sulfadimidine	Carp, rainbow trout	18-57	100-200 mg/kg sd	IV/PO	10-20
Sulfamethoxypyridazine	Rainbow trout	72	200 mg/kg sd	PO	13
Sulfamonomethoxine	Rainbow trout, yellowtail	5-33	100-400 mg/kg sd	IV/PO	15-22
Sulfanilamide	Rainbow trout	36	200 mg/kg sd	PO	13
Sulfathiazole	Rainbow trout	60	200 mg/kg sd	PO	13
Thiamphenicol	Sea bass	21	30 mg/kg 5d	PO	19
Tobramycin	Brown shark	48	1-2.5 sd	IM	25
Trimethoprim	Atlantic salmon, carp, rainbow trout	21-50	1-100 mg/kg sd	IV/PO	8-24
Vetoquinol	Cod	79	25 mg/kg sd	PO	8
	Atlantic salmon	16	40 mg/kg sd	PO	10

*sd, single dose.

treatments to try to improve permeability across the gills. Drug absorption and elimination can be affected by salinity under normal conditions, and the effects of hyperosmotic treatments have not been adequately studied. Certain drugs which bind divalent cations (such as the tetracyclines) may have their bioavailability compromised by the addition of salts. Ultrasound treatments to enhance absorption may be feasible in a small aquarium setting but have not been studied extensively. Both hyperosmotic and ultrasound treatments are fairly stressful. They are mainly used for vaccination rather than antimicrobial treatment (Treves-Brown, 2000; Navot et al., 2004).

Oral Treatments

Oral treatments are the most feasible methods for large commercial aquaculture systems because they are the least stressful for the animals. However, sick fish may not eat. This was recently demonstrated in a study where the concentration of oxolinic acid was examined in Atlantic salmon treated during an outbreak of winter ulcer disease (*Moritella viscosa*). Oxolinic acid was detected in plasma and tissues of healthy fish, whereas levels were below the limit of detection in moribund and dead fish (Coyne et al., 2004a). The moribund and dead fish also had no food in their gastrointestinal tracts. These results indicate that the antimicrobial appears to help actively feeding healthy fish fight off the infection, whereas fish with clinical signs are anorexic and therefore not receiving the antimicrobial. Variable intake can also occur if fish vary in size. The larger fish will probably consume more of the medicated feed than their smaller and less vigorous counterparts. Palatability, especially of the sulfa products, can also be a problem.

Absorption from the intestinal tract may vary from species to species. As mentioned previously, saltwater fish will drink and, therefore, drugs may bind cations in the water in their intestinal tracts, affecting bioavailability. The formulation of the drug may either enhance or decrease absorption.

Various methods for administering oral medication include commercial medicated feed, custom surface-coated feeds, custom feeds (e.g., gelatin diets), medicated live feeds (e.g., artemia grown in or fed antimicrobials), injecting food (e.g., small fish used for food) and tube feeding (Noga, 1996; Treves-Brown, 2000). Obviously, some of these techniques are appropriate only for pet/aquarium fish.

Injectable Treatments

Treating fish with injectable antimicrobials causes handling stress and can be a massive undertaking for commercial producers. Advantages include assuring that all animals receive the drug at the desired dose. Route of administration is often intramuscular, but sometimes intraperitoneal, intravascular, or intradorsal sinus (caudal to the dorsal fin) routes are used. Intramuscular treatments are usually administered in the epaxial muscles, above the lateral line and near the caudal fin. Since there is a renal portal vascular system in fish, it is best to inject aminoglycosides cranial to the dorsal fin to avoid large doses entering the kidney.

In pet/aquarium fish, most injections are given manually. Automatic injectors, such as those used in poultry operations, can be used in commercial aquaculture. Although this seems a formidable task, vaccinations are given by injection, often manually, to many net-pen reared fish (Noga, 1996; Vavarigos, 2003).

Avoiding Toxicity in the Target Animal

Even though fish drug metabolism can be affected by environmental salinity and temperature, it is remarkably similar to mammalian drug metabolism. Both groups have similar metabolic systems: phase 1 systems, including the heme protein monooxygenase (cytochrome P450) and the flavin monooxygenase (FMO) systems, and the phase 2 conjugation systems. The P450 systems have been identified in over 150 fish species (Whyte et al., 2000). FMO activity, however, is lacking in some fish species, e.g., channel catfish (Schlenk et al., 1995). This difference can affect the metabolism of drugs, resulting in either enhanced or reduced toxicity, depending on the chemical. For example, in the case of the herbicide aldicarb, catfish and trout take up similar amounts of the parent compound, but their metabolism of the compound differs (Perkins and Schlenk, 2000). Compared to trout, catfish are ten times less susceptible to toxicity induced by aldicarb because they lack FMO activity. Trout (like mammals) metabolize the parent drug to a toxic sulfoxide (Montesissa et al., 1995) that is responsible for most of the toxic effects (Perkins et al., 1999). Such differences in metabolism must be considered when choosing antimicrobials. In general, however, most antimicrobials given by the oral route will not cause toxicity because fish rarely overdose by eating excessive

amounts of medicated feed, and over-medicated feed is often not palatable and thus rejected.

Drugs administered via bath treatments can cause toxicity if grossly overdosed, especially if they are absorbed by the gills. In addition, saltwater fish will drink the water and thus probably get an oral dose as well. Drugs may have an effect on the water pH, which can affect the osmoregulation of the animal. High doses of tetracyclines, which are used for immersion treatments, can affect the pH of the water, inducing toxicity (Treves-Brown 2000). Drugs can also irritate the skin or gills. Waterborne irritants can affect the gills by increasing mucus production and thus decreasing gaseous exchange.

The main antimicrobial toxicity seen in fish is that of aminoglycoside-induced nephrotoxicosis. Aminoglycosides, such as gentamicin, which cannot be excreted through filtration in aglomerular fish (including toadfish, goosfish and seahorses), can cause extensive renal necrosis in these fish at doses that are therapeutic (and non-toxic) in other fish species (Reimschuessel et al., 1996). The half-life of gentamicin in toadfish is approximately two weeks, compared to two days in goldfish. Since fish eliminate nitrogenous waste through the gills, they can survive with compromised renal function as long as the osmolarity of their environment does not change dramatically. Since their kidneys can undergo nephron-neogenesis, fish can sometimes survive such a toxic episode and regenerate their kidneys. The risks and benefits of treatment must be carefully considered before using these antimicrobials.

Renal damage has also been associated with the use of erythromycin (Hick and Geraci, 1984) and sulfamerazine (Smith et al., 1973). A number of antimicrobials, including erythromycin, nalidixic acid, and sulfa drugs, can cause anorexia, especially if administered in high doses. Nalidixic acid and, to a lesser extent, oxolinic acid have induced macrocytic anemia, potentially due to their effect on DNA synthesis. Immunosuppression has been shown to occur with tetracyclines (Rijkers et al., 1980).

Ensuring Safety for Humans

Considerations for safety to humans include potential hazards associated with (1) administration of the drug, (2) exposure of individuals from environmental

contamination, and (3) consumption of the fish. Most hazards associated with administration of the drug can be managed by adequate training, specialized equipment, and personal protective clothing. Basic veterinary practices used to reduce hazards to personnel and the environment when treating terrestrial animals generally apply to treating fish.

Food safety concerns, for the most part, relate to residues of the drug used (or its metabolites) in the food product (Chapter 25). In order to prevent harmful residues, governmental agencies establish required withdrawal periods. These periods are designed to ensure that the food product will have residue levels below the "maximum residue levels" (MRLs) or "tolerances" established by the governing body. MRLs and tolerances are based on the potential toxicity of the compound and an assessment of potential exposure levels, including consideration of the general risk to the consumer. The basic principles are, again, similar to those used when treating terrestrial food animals. Withdrawal periods for fish, however, usually incorporate the temperature as part of the equation, sometimes in the form of "degree days" (the °C multiplied by the number of days, e.g., 50 days at 10 °C = 500 degree days, as does 25 days at 20 °C) (Alderman, 2000; EMEA, 2005; US FDA, 2005). The European Union (EU) regulations have included the concept of degree days in their suggested generic withdrawal period of 500 degree days for compounds for which no specific withdrawal period has been set. Knowledge of the pharmacokinetics and depuration patterns of different drugs in different fish species is essential for both those establishing such periods and clinicians using the drugs. When evaluating data reporting depuration periods and residue levels, one should also consider what detection method was used. Analytical methods have changed over the years, in general becoming more sensitive. As a result, residues being "below the level of detection" in the 1980s may actually be detected and considered unacceptable today. It is important for clinicians prescribing aquaculture drugs to be aware of the regulations in their country to protect the safety of the consumer.

Environmental Effects and Non-target Species

Treating fish with antimicrobials, especially in large commercial systems, can affect the environment in a

number of ways. These include: (1) toxicity to non-target species; (2) accumulation by non-target species; (3) accumulation in sediments; (4) presence in drinking water; and (5) alterations in the ecosystem's microbial community, including antimicrobial resistance. Local effluent discharge regulations must be considered both by the clinician and the owner of the aquaculture facility.

Toxicity to non-target species depends on the dose and the route of administration of the drug (Isidori et al., 2005). For example, furazolidone, which is usually administered by bath treatment, is extremely toxic to crustaceans (Macri et al., 1988). Accumulation of antimicrobials can occur in non-target species, including fish, crustaceans, and plants (Samuelsen et al., 1992; Delepee et al., 2003; Migliore et al., 2003). Accumulation in the sediment has also been documented for a number of antimicrobials, including flumequine, furazolidone, ormetoprim, oxolinic acid, and oxytetracycline (Bjorklund et al., 1991; Samuelsen et al., 1991; Capone et al., 1996; Lalumera et al., 2004). Antimicrobials and other pharmaceuticals, including sources from human and terrestrial agricultural use, have been detected in receiving waters (Hirsch et al., 1999; Kümmerer, 2001; Rooklidge, 2004). Finally, there has been recent concern about changes in antimicrobial susceptibility following antibiotic use (Samuelsen et al., 1992; Angulo, 1999; Guardabassi et al., 2000; Chelossi et al., 2003). Recent advances in standardizing methods for assessing antimicrobial susceptibility of aquatic pathogens should help efforts to monitor changes due to using antimicrobials in aquaculture systems (Miller et al., 2003, 2005; CLSI 2006a, 2006b).

Legal Considerations

Veterinarians dealing with food animals, either terrestrial or aquatic, must be familiar with the regulations regarding antimicrobial use in their country as well as in countries that may import the product (Chapter 26). These regulations include: (1) prohibitions from use, e.g., chloramphenicol (local and abroad); (2) residue tolerance levels, such as MRLs in the EU; (3) effluent and discharge regulations; (4) general prescription regulations.

Such laws vary greatly from country to country,

from almost no regulation to restrictive regulation. For example, the United States has only approved four antimicrobials—oxytetracycline, florfenicol, ormetoprim/sulfadimethoxine, and sulfamerazine—for use in aquatic food fish. Canada has approved the first three antimicrobials, as well as sulfadiazine and trimethoprim. Approximately ten antimicrobials have received authorization for use in certain EU member states, including quinolone antimicrobials such as flumequine, oxolinic acid, and sarafloxacin. Japan has approved approximately thirty antimicrobials for use in aquaculture (Treves-Brown, 2000; Schnick, 2001; US FDA, 2005). Many developing countries are beginning to formulate regulations regarding antimicrobial use in aquaculture.

Many countries are also developing provisions for using therapeutic agents that are not approved (extra- or off-label use) in minor species. In the United States, the US Food and Drug Administration (US FDA) lists some substances as “low regulatory priority”. Such substances include sodium chloride, sodium bicarbonate, and urea. Although not antimicrobials, these chemicals might be used in conjunction with other treatments. Also, the US Minor Use and Minor Species Animal Health Act (MUMS) provides regulatory authority to the US FDA (2004) to add certain drugs to an index of legally marketed but unapproved new animal drugs for use in minor species. MUMS provides more flexibility for veterinarians prescribing medicines to aquatic animals. The European Medicines Agency, which regulates antimicrobial use in the EU, is considering instituting similar policies (EMA 2005).

In addition to prescription regulations, many countries are developing guidelines for judicious use of antimicrobials in order to prevent antimicrobial resistance from developing in pathogens and environmental bacteria (Chapter 27). In the United States, such guidelines have been proposed by the American Veterinary Medical Association (AVMA 2002) and the National Aquaculture Association (2003). They are, in general, similar to guidelines proposed for antimicrobial use in terrestrial animals.

The clinician, then, must keep abreast of recent developments in both national and international regulations regarding antimicrobial use in aquatic species. The aquaculture producer must also be conversant in these areas.

Table 39.3. CLSI/NCCLS-approved disk diffusion zone diameter (mm) QC ranges for *Aeromonas salmonicida* subsp. *salmonicida* ATCC®25922. This also illustrates the effect of time and temperature of incubation on the size of the zone of inhibition (CLSI, 2006a).

Antimicrobial Agent	<i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i> ATCC®33658		
	22°C, 24-28 h	22°C, 44-48 h	28°C, 24-28h
Ampicillin	34-42	35-44	33-41
Enrofloxacin	36-46	37-49	35-45
Erythromycin	17-28	19-31	21-29
Florfenicol	32-44	34-47	33-41
Gentamicin	23-29	22-32	24-30
Ormetoprim/sulfadimethoxine	24-38	21-35	21-32
Oxolinic acid	34-43	33-45	32-41
Oxytetracycline	30-39	28-38	28-34
Trimethoprim/sulfamethoxazole	27-40	24-39	26-36

Antimicrobial Susceptibility Testing of Aquatic Bacteria

Defining in vitro conditions for antimicrobial susceptibility testing (AST) has been difficult because aquatic bacteria vary greatly in their optimal growth requirements. Temperature optimums of various aquatic bacteria can range from 15°C to 35°C. Some aquatic bacteria prefer or require supplementation to the basal medium, while others need a low-nutrient or diluted basal medium.

While temperature, salinity, and other preferences are important to consider when testing the antimicrobial susceptibility of an aquatic isolate, the ultimate goal of the test is to obtain a result that can be used to predict therapeutic efficacy. Standardized testing protocols are essential to obtain results that are reproducible within and among laboratories (Chapter 2). Such test protocols monitor performance and reproducibility using quality control (QC) and quality assurance (QA) parameters established by organizations like the CLSI and the Swedish Reference Group for Antibiotics.

CLSI has published two guidance documents, M42-P and M49-P, which describe two newly standardized methods for disk diffusion and broth dilution susceptibility testing of bacterial isolates from aquatic animals (CLSI 2006a, 2006b).

Disk Diffusion Susceptibility Testing

Although the Kirby-Bauer method is the most commonly used disk diffusion method in aquatic medicine, many studies have been published using different types of basal media for testing a cornucopia of aquatic pathogens (Bauer et al., 1966; Dalsgaard, 2001). Dalsgaard (2001) and Barker and Kehoe (1995) both found Mueller-Hinton agar to be the best medium for disk diffusion testing, based upon its consistent performance with a wide range of aquatic pathogens. An international collaborative study in 2003 conducted in accordance with existing CLSI guidelines (CLSI/NCCLS, 2002a) standardized the disk diffusion testing method for non-fastidious aquatic isolates that grow well on Mueller-Hinton agar (Miller et al., 2003). These aquatic bacteria have been labeled Group 1 isolates by the Aquaculture Working Group of the CLSI Subcommittee on Veterinary Antimicrobial Susceptibility Testing. Organisms in Group 1 prefer growth on Mueller-Hinton agar at 22°C or 28°C.

Disk diffusion zone diameter QC ranges were established for two control organisms, *Escherichia coli* ATCC®25922 and *Aeromonas salmonicida* subsp. *salmonicida* ATCC®33658, at both 22°C and 28°C (Table 39.3). The ranges were established for ampicillin, enrofloxacin, erythromycin, florfenicol, gentam-

Table 39.4. Potential modifications of standard methods for disk diffusion susceptibility testing of some fastidious pathogens of fish.

Organism	Medium	Incubation	Group
Enterobacteriaceae <i>Aeromonas salmonicida</i> (non-psychrophilic strains) <i>Aeromonas hydrophila</i> and other mesophilic <i>Aeromonads</i> <i>Pseudomonas</i> spp. <i>Plesiomonas shigelloides</i> <i>Shewanella</i> spp. Vibrionaceae and related bacteria (nonobligate halophilic strains)	MHA	22°C (24-28 h and/or 44-48 h) or 28°C (24-28 h)	Group 1 ^a
Vibrionaceae and Photobacteriaceae (obligate halophilic strains)	MHA + 1% NaCl	22°C (24-28 h and/or 44-48 h) or 28°C (24-28 h and/or 44-48 h)	Group 2
<i>Flavobacterium columnare</i>	Diluted MHA (1:7)	28 °C (24-28 h and/or 44-48 h)	Group 3
<i>Flavobacterium psychrophilum</i>	Diluted MHA ^b	15°C (44-48 h and/or 68-72 h)	Group 3
<i>Flavobacterium branchiophilum</i>			
<i>Lactococcus</i> spp.	MHA + 5% sheep blood	22°C (44-48 h + CO ₂ if necessary for growth)	Group 4A
<i>Vagococcus salmoninarum</i>			
<i>Streptococcus</i> spp., <i>Carnobacterium maltaromaticum</i> , and other streptococci	MHA + 5% sheep blood	28°C (24-28 h and/or 44-48 h + CO ₂ if necessary for growth)	Group 4B

^aOnly Group 1 organisms have a standardized disk diffusion susceptibility testing method.^bRecommended supplementation cannot be made at this time, but may include cations, horse or fetal calf serum, or NaCl.

icin, ormethoprim/sulfadimethoxine, oxolinic acid, oxytetracycline, and trimethoprim/sulfamethoxazole (Miller et al., 2003; CLSI, 2006a).

Aquatic pathogens in Groups 2-5 may require media other than Mueller-Hinton agar (Tables 39.4, 39.5). There are currently no QC parameters in place to control their tests. In these cases, the clinician should perform the following: (1) Identify the isolate. (2) Determine to which 'Group' the isolate belongs. (3) Test the isolate on the suggested media. (4) Use a QC organism under standardized conditions in parallel with the test isolate. (5) Determine if the test was within QC. (6) If a test result is out of QC, determine the cause and repeat as necessary.

Clinicians should also consult CLSI/NCCLS guideline M42-P, *Methods for Antimicrobial Disk Susceptibility Testing of Bacteria Isolated from Aquatic Animals* (CLSI, 2006a), for suggested conditions to test the more fastidious aquatic bacterial genera (Groups 2-5). Detailed instructions on the use of the disk diffusion method are available in this CLSI document.

Dilution Susceptibility Testing

Both agar and broth dilution AST methods are used in aquatic medicine. Results of dilution susceptibility

tests provide data in the form of a minimum inhibitory concentration (MIC), which has greater clinical relevance than a zone diameter value (Chapter 2). Advances in automated inoculation systems for broth microdilution susceptibility testing, discussed in Chapter 2, have fostered growing popularity in many aquatic animal medicine research laboratories.

A standardized broth dilution susceptibility testing method has been developed for non-fastidious aquatic organisms in Group 1 at 22°C and 28°C (Miller et al., 2005). Quality control ranges were established for ten antimicrobial agents against the same two QC strains used for disk diffusion tests in cation-adjusted Mueller-Hinton broth (Jorgensen et al., 1996; Jones et al., 2004) (Table 39.6). Clinicians should use CLSI/NCCLS guidance document M49-P, *Methods for Broth Dilution Susceptibility Testing of Bacteria Isolated from Aquatic Animals* (CLSI, 2006b).

The agar dilution method is also used to determine the MICs of many aquatic pathogens. Mueller-Hinton agar is always the preferred basal medium and appears to perform well with non-fastidious aquatic isolates (Ho et al., 2000; Tang et al., 2002). Supplements may be needed to test some fastidious organisms. Mueller-Hinton agar with NaCl (Samuelson et al., 2003; Coyne et al., 2004b),

Table 39.5. Potential modifications of standard methods for disk diffusion susceptibility testing of special problem pathogens of fish.

Organism	Medium	Incubation	Grouping
Psychrophilic <i>Aeromonas salmonicida</i> strains	MHA	15 °C (44–48 h)	Group 5
<i>Vibrio salmonicida</i> and <i>Moritella viscosa</i>	MHA with supplementation ^a	15 °C (6 days)	Group 5
<i>Tenacibaculum maritimum</i>	Diluted MHA (1:7) + inorganic ion supplementation ^b	25 °C (24–28 h)	Group 5
<i>Renibacterium salmoninarum</i>	Unknown ^a	15 °C	Group 5
<i>Mycobacterium</i> spp. and <i>Nocardia seriolae</i>	See CLSI/NCCLS document M24	See CLSI/NCCLS document M24	Group 5
<i>Erysipelothrix rhusiopathiae</i>	Chocolate MHA	35 °C	Group 5

^aRecommended supplementation cannot be stated at this time, but may include cations, horse or fetal calf serum, or NaCl.

^bSee CLSI/NCCLS document M42A.

Table 39.6. CLSI-approved broth dilution minimum inhibitory concentration (MIC) QC ranges (µg/mL) for *Aeromonas salmonicida* subsp. *salmonicida* ATCC®33658 (CLSI, 2005b).

Antimicrobial Agent	<i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i> ATCC®33658		
	22°C, 24–28h	22°C, 44–48h	28°C, 24–28h
Ampicillin	0.12 – 1	0.25 – 1	0.12 – 1
Enrofloxacin	0.008 – 0.03	0.008 – 0.03	0.004 – 0.03
Erythromycin	4 – 16	4 – 32	4 – 32
Florfenicol	0.25 – 1	0.5 – 2	0.5 – 2
Flumequine	0.015 – 0.12	0.03 – 0.12	0.015 – 0.12
Gentamicin	0.25 – 1	0.25 – 2	0.25 – 1
Ormetoprim / sulfadimethoxine	0.06/1.2 – 0.25/4.8	0.06/1.2 – 0.5/9.5	0.06/1.2 – 0.5/9.5
Oxolinic acid	0.008 – 0.03	0.008 – 0.03	0.008 – 0.03
Oxytetracycline	0.06 – 0.25	0.12 – 1	0.12 – 1
Trimethoprim / sulfamethoxazole	0.03/0.6 – 0.12/2.4	0.03/0.6 – 0.25/4.8	0.03/0.6 – 0.25/4.8

seawater (Torkildsen et al., 2000), horse serum (Michel et al., 2003) and sheep blood (McGinnis et al., 2003) have all been used. A diluted form of Mueller-Hinton agar based on a recommendation by Hawke and Thune (1992) has also been used in tests on the *Flavobacteria* (Bruun et al., 2000; Schmidt et al., 2000).

The agar dilution method is considered the 'gold' or reference standard for dilution susceptibility testing in mammals. However, because no QC ranges are currently available for use in tests conducted at temperatures less than 35°C, we recommend using broth dilution tests until standardized methods are available.

Susceptibility Breakpoints for Aquatic Bacterial Pathogens

Currently, no susceptibility test breakpoints (susceptible, intermediate, or resistant) or interpretive criteria are established for any aquatic pathogen against any

antimicrobial in any fish species. Clinicians are often expected to rely on their own experience and published data to make judgment calls on the interpretation of AST data. Clinical breakpoints are critical values that should be specific for a particular pathogen and can be used to predict therapeutic efficacy in the host (Chapter 2). Because fish, unlike terrestrial animals, are reared in heterogeneous environments that can drastically alter depuration rates and drug absorption, the pharmacokinetics/pharmacodynamics (PK/PD) for a given antimicrobial may vary greatly. This has made it difficult for researchers to include PK/PD data when attempting to set clinical breakpoints for aquatic pathogens (Coyne et al., 2004b). Most pharmacokinetic data have been obtained from studies of healthy fish in laboratory situations. It will be important to correlate these data with studies conducted under clinical conditions.

Integrated pharmacokinetic and pathogen susceptibility data are used both in designing dosage regimens and setting clinical breakpoints. PK/PD assessments help clinicians choose the appropriate antimicrobial agent and develop new dosing regimes targeted for specific species with specific diseases (Maglio and Nicolau, 2004). How breakpoints are derived has been discussed extensively in Chapter 2. There is considerable work required to define breakpoints, which will require a coordinated effort from both clinicians treating aquatic animals and the research community.

Bibliography

- American Fisheries Society. 2005. AFS Bluebook (CD-ROM): Suggested procedures for the detection and identification of certain finfish and shellfish pathogens. Bethesda, MD: American Fisheries Society.
- Alderman DJ. 2000. Antimicrobial drug use in aquaculture. In: Prescott JE, Baggot JD, Walker RD., eds. Antimicrobial Therapy in Veterinary Medicine, 3rd ed. Ames, IA: Blackwell Publishing.
- Angulo F. 1999. Use of antimicrobial agents in aquaculture: potential for public health impact. *J Agric Food Chem* 48:1673.
- American Veterinary Medical Association (AVMA). 2002. Judicious and prudent antimicrobial drug use principles for food fish veterinarians. <http://www.avma.org/scientact/jtua/fish/jtuaafish.asp>.
- Barker GA, Kehoe E. 1995. Assessment of disc diffusion methods for susceptibility testing of *Aeromonas salmonicida*. *Aquaculture* 134:1.
- Bauer AW, et al. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 45:493.
- Benbrook C. 2002. Antibiotic drug use in U.S. aquaculture. <http://www.mindfully.org/Water/Antibiotic-Aquaculture-BenbrookFeb02.htm>.
- Bjorklund HV, Bylund G. 1991. Comparative pharmacokinetics and bioavailability of oxolinic acid and oxytetracycline in rainbow trout (*Oncorhynchus mykiss*). *Xenobiotica* 21:1511.
- Bowser PR, et al. 1992. Pharmacokinetics of enrofloxacin in fingerling rainbow trout (*Oncorhynchus mykiss*). *J Vet Pharmacol Ther* 15:62.
- Bruun MS, et al. 2000. Antimicrobial resistance patterns in Danish isolates of *Flavobacterium psychrophilum*. *Aquaculture* 187:201.
- Capone DG, et al. 1996. Antibacterial residues in marine sediments and invertebrates following chemotherapy in aquaculture. *Aquaculture* 145:55.
- Carpenter JW. 2005. Exotic Animal Formulary, 3rd ed. St. Louis: Elsevier Saunders.
- Chelossi E, et al. 2003. Antibiotic resistance of benthic bacteria in fish-farm and control sediments of the Western Mediterranean. *Aquaculture* 219:83.
- Chen CY, et al. 2004. Oxytetracycline residues in four species of fish after 10-day oral dosing in feed. *J Aquatic Animal Health* 16:208.
- Clinical Laboratory Standards Institute (CLSI/NCCLS). 2002a. Development of in vitro susceptibility testing criteria and quality control parameters for veterinary antimicrobial agents, Approved Guideline M37-A2. Wayne, Pa: CLSI.
- CLSI/NCCLS. 2002b. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, Approved Standard M31-A2. Wayne, Pa: CLSI.
- CLSI/NCCLS. 2006a. Methods for antimicrobial disk susceptibility testing of bacteria isolated from aquatic animals, Proposed Guideline M42-P. Wayne, Pa: CLSI.
- CLSI/NCCLS. 2006b. Methods for broth dilution susceptibility testing of bacteria isolated from aquatic animals, Approved Guideline M49-P. Wayne, Pa: CLSI.
- Coyne R, et al. 2004a. A comparison of oxolinic acid concentrations in farmed and laboratory held rainbow trout (*Oncorhynchus mykiss*) following oral therapy. *Aquaculture* 239:1.
- Coyne R, et al. 2004b. Attempt to validate breakpoint MIC values estimated from pharmacokinetic data obtained during oxolinic acid therapy of winter ulcer disease in Atlantic salmon (*Salmo salar*). *Aquaculture* 238:51.
- Dalsgaard I. 2001. Selection of media for antimicrobial susceptibility testing of fish pathogenic bacteria. *Aquaculture* 196:267.
- Delepee R, et al. 2003. The bryophyte *Fontinalis antipyretica* Hedw. bioaccumulates oxytetracycline, flumequine and oxolinic acid in the freshwater environment. *Sci Total Environ* 322:243.
- Elema MO, et al. 1996. Bioavailability of oxytetracycline from medicated feed administered to Atlantic salmon (*Salmo salar* L.) in seawater. *Aquaculture* 143:7.
- Elston RA, et al. 1994. Comparative uptake of orally administered difloxacin in Atlantic salmon in freshwater and seawater. *J Aquatic Animal Health* 6:341.
- European Medicines Agency (EMA). 2005. About the EMA. <http://www.emea.eu.int/htmls/aboutus/emeaoverview.htm>.
- Food and Agriculture Organization of the United Nations (FAO). 2003. Assessment and management of seafood safety and quality. http://www.fao.org/documents/show_cdr.asp?url_file=/DOCREP/006/Y4743E/y4743e0e.htm.
- Guardabassi L, et al. 2000. Increase in the prevalence of oxolinic acid resistant *Acinetobacter* spp. observed in a stream receiving the effluent from a freshwater trout farm following the treatment with oxolinic acid medicated feed. *Aquaculture* 188:205.
- Hawke JP, Thune RL. 1992. Systemic isolation and antimicrobial susceptibility of *Cytophaga columnaris* from commercially reared channel catfish. *J Aquatic Animal Health* 4:109.
- Hick BD, Geraci JR. 1984. A histological assessment of damage in rainbow trout, *Salmo gairdneri* Richardson fed rations containing erythromycin. *J Fish Dis* 7:457.
- Ishida N. 1992. Tissue levels of oxolinic acid after oral and intravascular administration to freshwater and seawater rainbow trout. *Aquaculture* 102:9.

- Isidori M, et al. 2005. Toxic and genotoxic evaluation of six antibiotics on non-target organisms. *Sci Total Environ* 346:87.
- Joint Subcommittee on Aquaculture. 2004. Guide to drug, vaccine and pesticide use in aquaculture. <http://aquanic.org/jsa/wgqaap/drugguide/drugguide.htm>.
- Jones J, et al. 1997. Gentamicin concentrations in toadfish and goldfish serum. *J Aquatic Animal Health* 9:211.
- Jones RN, et al. 2004. Validation of commercial dry-form broth microdilution panels and test reproducibility for susceptibility testing of dalbavancin, a new very long-acting glycopeptide. *Int J Antimicrob Agents* 23:197.
- Jorgensen JH, et al. 1996. Development of interpretive criteria and quality control limits for macrolide and clindamycin susceptibility testing of *Streptococcus pneumoniae*. *J Clin Microbiol* 34:2679.
- Kümmerer K. 2001. Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources—a review. *Chemosphere* 45:957.
- Lalumera GM, et al. 2004. Preliminary investigation on the environmental occurrence and effects of antibiotics used in aquaculture in Italy. *Chemosphere* 54:661.
- MacMillan, JR. 2003. Drugs used in the US aquaculture industry. Aquaculture Network Information Center (AquaNIC), http://aquanic.org/asap/white_pages/drugs.pdf.
- MacMillan JR, et al. 2003. Volume of antibiotics sold (2001 and 2002) in US domestic aquaculture industry. <http://www.nationalaquaculture.org/pdf/AETF%20Antibiotic%20use%20white%20paper%206.11.03.pdf>.
- Macri A, et al. 1988. Acute toxicity of furazolidone on *Artemia salina*, *Daphnia magna*, and *Culex pipiens* molestus larvae. *Ecotoxicol Environ Saf* 16:90.
- Maglio D, Nicolau DP. 2004. The integration of pharmacokinetics and pathogen susceptibility data in the design of rational dosing regimens. *Methods Find Exp Clin Pharmacol* 26:781.
- Martinsen B, et al. 1993a. Single dose pharmacokinetic study of florfenicol in Atlantic salmon (*Salmo salar*) in seawater at 11°C. *Aquaculture* 112:1.
- McGinnis A, et al. 2003. In vitro evaluation of the susceptibility of *Edwardsiella ictaluri*, etiological agent of enteric septicemia in channel catfish, *Ictalurus punctatus* (Rafinesque), to florfenicol. *J Vet Diagn Invest* 15:576.
- Michel C, et al. 2003. Chloramphenicol and florfenicol susceptibility of fish-pathogenic bacteria isolated in France: comparison of minimum inhibitory concentration, using recommended provisory standards for fish bacteria. *J Appl Microbiol* 95:1008.
- Migliore L, et al. 2003. Phytotoxicity to and uptake of enrofloxacin in crop plants. *Chemosphere* 52:1233.
- Miller RA, et al. 2003. Antimicrobial susceptibility testing of aquatic bacteria: Quality control disk diffusion ranges for *Escherichia coli* ATCC 25922 and *Aeromonas salmonicida* subsp. *salmonicida* ATCC 33658 at 22 and 28 degrees C. *J Clin Microbiol* 41:4318.
- Miller RA, et al. 2005. A standardized method for the determination of minimum inhibitory concentrations of aquatic bacteria using *Escherichia coli* ATCC 25922 and *Aeromonas salmonicida* subsp. *salmonicida* ATCC 33658 as quality control organisms at 22°C, 28°C, and 35°C. *Dis Aquat Organ* 64:211.
- Montesissa C, et al. 1995. In vitro comparison of aldicarb oxidation in various food-producing animal species. *Vet Hum Toxicol* 37:333.
- National Aquaculture Association. 2003. Judicious antimicrobial use in US aquaculture: principles and practices. <http://www.nationalaquaculture.org/pdf/Judicious%20Antimicrobial%20Use.pdf>.
- Navot N, et al. 2004. Enhancement of antigen uptake and antibody production in goldfish (*Carassius auratus*) following bath immunization and ultrasound treatment. *Vaccine* 22:2660.
- Noga EJ. 1996. Fish Disease, Diagnosis and Treatment. St. Louis: Mosby-Year Book.
- Office International des Epizooties (OIE). 2003. Manual of Diagnostic Tests for Aquatic Animals. http://www.oie.int/fr/normes/fmanual/A_index.htm.
- Ornamental Aquatic Trade Association (OATA). 1999. Antibiotics in the ornamental fish industry. <http://www.aquaticsworldwide.org/archive/issue2/antibiotics.htm>.
- Perkins EJ Jr., et al. 1999. In vitro sulfoxidation of aldicarb by hepatic microsomes of channel catfish, *Ictalurus punctatus*. *Toxicol Sci* 48:67.
- Perkins EJ Jr., Schlenk D. 2000. In vivo acetylcholinesterase inhibition, metabolism and toxicokinetics of aldicarb in channel catfish: role of biotransformation in acute toxicity. *Toxicol Sci* 53:308.
- Reimschuessel R, et al. 1996. Evaluation of gentamicin-induced nephrotoxicosis in the toadfish, *Opsanus tau*. *J Am Vet Med Assoc* 209:137.
- Reimschuessel R, et al. 2005. Fish drug analysis—Phish-Pharm: A searchable database of pharmacokinetics data in fish. *Am Assn Pharm Sci J* 07(02):E288-E327. <http://www.aapsj.org/view.asp?art=aapsj070230>.
- Rijkers GT, et al. 1980. The immune system of cyprinid fish: the immunosuppressive effect of the antibiotic oxytetracycline in carp (*Cyprinus carpio* L.). *Aquaculture* 19:177.
- Rogstad A, et al. 1991. Pharmacokinetic study of oxytetracycline in fish. I. Absorption, distribution and accumulation in rainbow trout in freshwater. *Aquaculture* 96:219.
- Rooklidge SJ, 2004. Environmental antimicrobial contamination from terraccumulation and diffuse pollution pathways. *Sci Total Environ* 325:1.
- Samuelsen OB, et al. 1991. Fate and microbiological effects of furazolidone in a marine aquaculture sediment. *Sci Total Environ* 108:275.
- Samuelsen OB, et al. 1992a. Residues of oxolinic acid in wild fauna following medication in fish farms. *Dis Aquat Organ* 12:111.
- Samuelsen OB, et al. 1992b. Long-range changes in oxytetracycline concentration and bacterial resistance towards oxytetracycline in a fish farm after medication. *Sci Total Environ* 114:25.
- Schnick RA. 2001. Aquaculture chemicals. In: Kirk-Othmer Encyclopedia of Chemical Technology, 4th ed, V3. New York, NY: John Wiley and Sons, Inc. Online database: <http://www2.lib.udel.edu/database/kirkothmer.html>.

- Schlenk D, et al. 1995. Differential expression and activity of flavin-containing monooxygenases in euryhaline and stenohaline flatfishes. *Comp Biochem Physiol C* 112:179-186.
- Schmidt AS, et al. 2000. Occurrence of antimicrobial resistance in fish—pathogenic and environmental bacteria associated with four Danish rainbow trout farms. *Appl Environ Microbiol* 66:4908.
- Smith CE, et al. 1973. Sulphamerazine toxicity in cutthroat trout brood fish (*Salmo clarki*). *J Fish Biology* 5:97-101.
- Sohlberg S, et al. 1990. Plasma concentrations of flumequine after intra-arterial and oral administration to rainbow trout (*Salmo gairdneri*) exposed to low water temperatures. *Aquaculture* 84:355.
- Sohlberg S, et al. 1994. Temperature-dependent absorption and elimination of flumequine in rainbow trout (*Oncorhynchus mykiss* Walbaum) in fresh water. *Aquaculture* 119:1.
- Stoskopf MK, et al. 1986. Therapeutic aminoglycoside antibiotic levels in brown shark, *Carcharhinus plumbeus* (Nardo). *J Fish Dis* 9:303.
- Stoskopf MK. 1993. Chemotherapeutics. In: Stoskopf MK, ed. *Fish Medicine*. Philadelphia: WB Saunders.
- Stoskopf MK. 1999. Fish pharmacotherapeutics. In Fowler ME, Miller RE, eds. *Zoo & Wild Animal Medicine: Current Therapy* 4. Philadelphia: WB Saunders.
- Struhsaker JW, et al. 1973. Effect of antibiotics on survival of carangid fish larvae (*Caranx mate*), reared in the laboratory. *Aquaculture* 2:53.
- Torkildsen L, et al. 2000. Minimum inhibitory concentrations of chloramphenicol, florfenicol, trimethoprim/sulfadiazine and flumequine in seawater of bacteria associated with scallops (*Pecten maximus*) larvae. *Aquaculture* 185:1.
- Treves-Brown KM. 2000. *Applied Fish Pharmacology*. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- United States Food and Drug Administration (US FDA) Center for Veterinary Medicine. 2005. *Aquaculture*. <http://www.fda.gov/cvm/aqualibtoc.htm>.
- US FDA Center for Veterinary Medicine & US Department of Agriculture CSREES. 2004. National Research Support Project 7 (NRSP-7). International workshop minor-use and minor species: a global perspective. Rockville, MD: US FDA. <http://www.fda.gov/cvm/Fall04MUMSPresentations.htm>.
- US FDA. 1997. Extralabel animal drug use; fluoroquinolones and glycopeptides; order of prohibition. *Federal Register* 62(99):27944-947. <http://www.fda.gov/cvm/fqnotice.htm>.
- Vavarigos P. 2003. Immersion or injection? Practical considerations of vaccination strategies. http://www.vetcare.gr/Fish_Vaccination_Strategies.htm#injection.
- Whyte JJ, et al. 2000. Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. *Crit Rev Toxicol* 30:347.

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